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#### (54)Interferon gamma inducing factor

(57)Disclosed are created stable polypeptides which are capable of inducing the production of interferon-gamma by immunocompetent cells. The present polypeptides contain specific amino acid sequences usually derived from the wild-type polypeptides, being capable of the production of interferon-gamma, by replacing the cysteine(s) with different amino acid(s). The present polypeptides possess a stability and an activity of inducing the production of IFN-γ by immunocompetent cells, both of which are significantly higher than those of the wild-type polypeptides. In addition to the activity, the present polypeptides can exhibit remarkable activities of inducing the formation of killer cells and enhancing thier cytotoxicities. The present polypeptides are easily obtainable by the process according to the present invention using recombinant DNA techniques. Thus the present polypeptides are useful for agents to treat and/or prevent susceptive diseases such as viral diseases, infections, malignant tumors, and immunopathies.

### Description

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The present invention relates to novel biologically active polypeptides, more particularly, artificially created polypeptides which are commonly capable of inducing the production of interferon-gamma (hereinafter abbreviated as "IFN- $\gamma$ ") by immunocompetent cells.

The present inventors successfully isolated some polypeptides which are capable of inducing the production of IFN-y by immunocompetent cells, as well as cloned cDNAs which encode the polypeptides (hereinafter called "the wild-type polypeptides"); the relating inventions are disclosed in Japanese Patent Kokai Nos.27, 189/96 and 193,098/96 and Japanese Patent Application No.67,434/96. It is known that the wild-type polypeptides usually contain SEQ ID NOs:1-3 as consensus partial amino acid sequences, and that they usually possess properties of inducing the formation of killer cells and enhancing their cytotoxicities, in addition to the property of inducing production of IFN-γ, a useful biologically active protein. Because of the properties, the use of the wild-type polypeptides as antiviral, antimicrobial, antitumor, and/or anti-immunopathic agents, etc. is in great expectation.

However, as described in Japanese Patent Application No.67,434/96 by the present applicant, there is the problem that natural cells commonly do not enable producing the wild-type polypeptide in desired amounts. The present inventors energetically investigated the cause, revealing that the wild-type polypeptides usually exist in the form of precursor exhibiting no biological activity in natural cells. The precursor has been proved to be converted into an active form by the action of converting enzymes such as interleukin-1β converting enzymes, which is described in Japanese Patent Application Nos.207,691/96 and 213,267/96 by the present applicant.

The wild-type polypeptides are probably instable, which would be involved in the above problem as another cause. Progress in recombinant DNA techniques of recent years have facilitated to remove and/or replace one or more amino acids in biologically active proteins to develop mutagenized polypeptides. However, even the progressed recombinant DNA techniques couldn't improve the stability of every protein with the inherent activity, unless taking trails and errors on the proteins individually.

In view of the foregoing, the first object of the present invention is to provide a polypeptide with significantly improved stability, while substantially retaining a biological activity of the wild-type polypeptide.

The second object of the present invention is to provide a process for producing the polypeptide.

The third object of the present invention is to provide a use of the polypeptide for a pharmaceuticals.

The present inventors energetically studied to attain the above objects, revealing that a polypeptide is more stable than the wild-type polypeptide, wherein the stale polypeptide contain an amino acid sequence derived either from a polypeptide containing the partial amino acid sequences of SEQ ID NOs:1-3 by replacing one or more of the cysteines with a different amino acid(s), or from the cysteine-replaced amino acid sequences by adding, removing and/or replacing one or more amino acids to and/or at position(s) excepting the position(s) where the cysteine(s) has been replaced; and that some of the stable polypeptides, in which the cysteine(s) have been replaced, exhibit an activity of inducing the production of IFN-y by immunocompetent cells significantly higher than the wild-type polypeptides. These polypeptides proved to be easily produced in a desired amount by using recombinant DNA techniques and to exhibit less toxicities. Based on the above, the present polypeptides were confirmed to be effectively used not only as an IFN-y inducer but also as a pharmaceutical.

The first object of the present invention is attainable by an isolated polypeptide which is capable of inducing the production of interferon-gamma by immunocompetent cells, said polypeptide containing either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs:1-3 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine.

The second object of the present invention is attainable by a process for producing a polypeptide, which comprises the steps of culturing a cell containing a DNA encoding the present polypeptide to produce a polypeptide, and collecting the produced polypeptide from the resulting culture.

The third object of the present invention is attainable by an agent for susceptive diseases, which contains the present polypeptide as an effective ingredient.

- FIG. 1 is the restriction map of a recombinant DNA "pCSHIGIF/MUT12" encoding a polypeptide according to the present invention.
- FIG. 2 is the restriction map of a recombinant DNA \*pCSHIGIF/WT\* encoding the wild-type polypeptide from human origin.
- FIG. 3 shows the time course of activity upon incubation of the polypeptides according to the present invention and the wild-type polypeptide, from human origin.
- FIG. 4 is the restriction map of a recombinant DNA "pCSHIGIF/MUT21" encoding another polypeptide according to the present invention.
- FIG. 5 is the restriction map of a recombinant DNA "pCSHIGIF/MUT25" encoding further another polypeptide according to the present invention.

- FIG. 6 is the restriction map of a recombinant DNA "pCSHIGIF/MUT32" encoding further another polypeptide according to the present invention.
- FIG. 7 is the restriction map of a recombinant DNA "pCSHIGIF/MUT41" encoding further another polypeptide according to the present invention.
- FIG. 8 is the restriction map of a recombinant DNA "pCSHIGIF/MUT35" encoding further another polypeptide according to the present invention.
- FIG. 9 is the restriction map of a recombinant DNA "pCSHIGIF/MUT42" encoding further another polypeptide according to the present invention.
- FIG. 10 is the restriction map of a recombinant DNA "pCSMIGIF/MUT11" encoding further another polypeptide according to the present invention.
- FIG. 11 is the restriction map of a recombinant DNA "pCSMIGIF/WT" encoding the wild-type polypeptide from mouse origin.
- FIG. 12 shows the time course of activity upon incubation of the polypeptides according to the present invention and the wild-type polypeptide, from mouse origin.
- FIG. 13 is the restriction map of a recombinant DNA "pCSMIGIF/MUT12" encoding further another polypeptide according to the present invention.

### [Explanation of Symbols]

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The symbol "CMV" indicates a cytomegalovirus promoter.

The symbol "IFNss" indicates a nucleotide sequence encoding the signal peptide of the subtype  $\alpha$ 2b of human interferon- $\alpha$ .

The symbols "IGIF/WT" and "mIGIF/WT" indicate cDNAs encoding any one of the wild-type polypeptides.

The symbols of "IGIF/MUT12", "IGIF/MUT21", "IGIF/MUT25", "IGIF/MUT32", "IGIF/MUT32", "IGIF/MUT41", "IGIF/MUT35", "IGIF/MUT42", "mIGIF/MUT11" and "mIGIF/MUT12" indicate cDNAs each of which encodes one of the polypeptides according to the present invention.

The followings are preferred embodiments according to the present invention. The polypeptides according to the present invention include all of the polypeptides which is capable of inducing production of interferon-gamma by immunocompetent cells, wherein said polypeptides contain either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs:1-3 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine. The different amino acids to replace the cysteine(s) are not restricted to any types, as far as the resulting polypeptide, containing an amino acid sequence replaced with the different amino acid(s), exhibits an activity of inducing production of IFN-γ by immunocompetent cells in the presence or absence of an appropriate cofactor, as the wild-type polypeptides containing SEQ ID NOs:1-3 as consensus partial amino acid sequences, and a stability significantly higher than that of the wild-type polypeptides. The different amino acids include serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine, among which the most preferable amino acid is serine or alanine. Embodiments of the amino acid sequences, containing SEQ ID NOs:1-3 as consensus partial amino acid sequences, in which one or more cysteines are to be replaced with different amino acid(s) are the wild-type polypeptides containing the SEQ ID NO:4 or 5. The SEQ ID NO:4 contains cysteines at the 38th, 68th, 76th, and 127th positions from the N-terminus. The SEQ ID NO:5 contains cysteines at the 7th, 75th, and 125th positions.

The present polypeptides include those containing the amino acid sequence of any one of SEQ ID NOs:6-12, which are derived from the wild-type polypeptide containing SEQ ID NO:4, those containing the amino acid sequence of SEQ ID NO:13 or 14, which are derived from the wild-type polypeptide containing the amino acid sequence of SEQ ID NO:5, and those containing an amino acid sequence derived from any one of SEQ ID NOs:6-14 by adding, removing, and/or replacing one or more amino acids to and/or at position(s) excepting the positions where the cysteine(s) have been replaced while retaining the desired biological activities and stability. The wording "one or more amino acids" means the number of amino acids which conventional methods such as site-directed mutagenesis can usually add, remove or replace. The polypeptides containing any one of SEQ ID NOs:6-14 possess both stability and biological activities significantly higher than those of the wild-type polypeptides.

The present polypeptides can be produced by recombinant DNA techniques of: transforming appropriate host cells with DNAs encoding the present polypeptides to obtain a cell containing the DNAs, culturing the cells containing the DNAs to produce the polypeptides, and collecting the produced polypeptides from the resulting culture. The present invention additionally provides a process using the recombinant DNA techniques for producing the present polypeptides, by which the present polypeptides can be easily obtained in a desired amount.

The DNAs used in the present process include all of the DNAs encoding any one of the present polypeptides, which can be obtained by a method of either artificial mutagenesis of DNAs from natural sources or chemical synthesis.

An example of the former method is as follows: preparing a DNA with the nucleotide sequence of SEQ ID NO:25 or 28 encoding the amino acid sequence of SEQ ID NO:4 or 5, respectively, from a natural cell as a source, and then applying "overlap extension", a method reported in Robert M. Horton et al. *Methods in Enzymology*, Vol.217 (New York: Academic Press, Inc., 1993), pp.270-279, to the DNA to replace one or more codons for the cysteines in SEQ ID NO: 4 or 5 with codon(s) for different amino acid(s). The present DNAs include DNAs containing any one of the nucleotide sequence of SEQ ID NOs:15-21, derived from SEQ ID NO:25, SEQ ID NOs:22 and 23, derived from SEQ ID NO:28, the complementary nucleotide sequences to SEQ ID NOs:15-23, and others derived from these nucleotide sequences by replacing one or more of the nucleotides with different one(s) without altering the amino acid sequences encoded thereby. An example of the latter method is chemical synthesis, by which the present DNAs are obtainable in usual manner based on the nucleotide sequences of SEQ ID NOs:9-15. Once obtained by any method, the present DNAs can be easily amplified to a desired amount by using PCR.

Generally in this field, when allowing a DNA encoding a polypeptide to express in a host cell, to improve the expressing efficiency or the biological activities of the polypeptide expressed, one or more nucleotides in the DNA can be replaced with different ones, and an appropriate promoter(s) and/or enhancer(s) can be linked to the DNA. The present DNAs can be also altered similarly as such. For example, nucleotide sequences for recognition sites by appropriate restriction enzymes, initiation codons, termination codons, and/or appropriate signal peptides including the signal peptide of the subtype a2b of interferon-α, shown in SEQ ID NO:16, can be arbitrary linked to the 5'- and/or 3'-termini of any of the nucleotide sequences of SEQ ID NOs:9-15, unless the resulting polypeptides diminish the desired biological activities and stabilities.

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The present DNAs can express the present polypeptides with improved stabilities and biological activities after introduced into appropriate host cells from microbial, vegetal, or animal origin, preferably, animal origin. The present DNAs can be introduced into the host cells in the form of recombinant DNAs. The recombinant DNAs usually comprise one of the present DNAs and one of autonomously replicable vectors, which are obtainable by conventional recombinant DNA techniques, once the present DNAs are obtained. Embodiments of the vectors into which the present DNAs can be inserted are plasmid vectors including pCD, pCDL-SRα, pKY4, pCDM8, pCEV4 and pME18S, which usually comprise nucleotide sequences suitable for expressing the present DNAs in hosts, e.g., promotors, enhancers, replication origins, terminators of transcription, splicing sequences, and/or selection markers. The use of vectors with a promotor such as a heat shock protein promotor or the interferon-α promotor disclosed by the present applicant in Japanese Patent Kokai No. 163,368, enables to regulate the present DNAs expression in the transformats by external stimuli.

To insert the present DNAs into the vectors, any conventional method in this field can be used. For example, DNAs containing the present DNAs and the vectors as above are digested by restriction enzymes and/or ultrasonication before the resulting fragments from the present DNAs are ligated with the vector fragments. Digestion by the restriction enzymes, which act on specific nucleotides, preferably, Acd, BamHI, BgMI, BstXI, EcoRI, HindIII, NotI, PstI, Sad, SalI, Smal, Spel, Xbal, XhoI, etc., facilitate to ligate the DNA fragments with the vector fragments. When ligating the DNA fragments with the vector fragments, they are, if necessary, first annealed, and then treated with a DNA ligase in vivo or in vitro. The recombinant DNAs thus obtained can be unlimitedly replicated in hosts from microbial or animal origin.

The recombinant DNAs can be introduced into host cells suitable to produce the present polypeptides. Whereas any cells conventionally used as host cells in this field can be used in the present invention, the host cells from yeast or mammalian origin are more preferable when the polypeptides produced are used for pharmaceuticals. Embodiments of the host cells from mammalian origin are epithelial, interstitial, and hemopoietic cells from human, monkey, mouse, and hamster, which include 3T3 cells, C127 cells, CHO cells, CV-1 cells, COS cells, HeLa cells, MOP cells, and their mutants. To introduce the present DNAs into the hosts, any conventional methods can be used, e.g., DEAE-dextran method, calcium phosphate transfection method, electroporation method, lipofection method, microinjection method, and viral infection method as using retrovirus, adenovirus, herpesvirus, and vaccinia virus, etc. To select clones producing the present polypeptides from the transformants, the transformants can be cultured before examining the resulting cultures for the present polypeptides produced. The recombinant DNA techniques using mammalian host cells are detailed in publications such as Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA eds., *Jikken-Igaku-Bessatsu*, *Saibo-Kogaku Handbook* (The handbook for the cell engineering), (Tokyo, Japan: Yodosha. Co., Ltd., 1992), and Takashi YOKOTA and Kenichi ARAI eds., *Jikken-Igaku-Bessatsu*, *Biomanual Series 3*, *Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), (Tokyo, Japan: Yodosha Co., Ltd., 1993).

The transformants thus obtained, cells containing the present DNAs, can produce the present polypeptides intracellularly and/or extracellularly when cultured in culture media. For the culture media, any conventional ones used for transformants can be used. The culture media generally comprise: buffers as a base; inorganic ions such as sodium ion, potassium ion, calcium ion, phosphoric ion, and chloric ion; micronutrients, carbon sources, nitrogen sources, amino acids and vitamins, which can be used depending on metabolic abilities of the cells; and sera, hormones, cell growth factors, and cell adhesion factors, which are used if necessary. Examples of the culture media are 199 medium, DMEM medium, Ham's F12 medium, IMDM medium, MCDB 104 medium, MCDB 153 medium, MEM medium, RD

medium, RITC 80-7 medium, RPMI-1630 medium, RPMI-1640 medium, and WAJC 404 medium. Culturing the present transformants under the following conditions can generate cultures containing the present polypeptides: inoculating the present transformants into the culture media to give a cell density of 1 x  $10^4$  - 1 x  $10^7$  cells/ml, more preferably, 1 x  $10^5$  - 1 x  $10^6$  cells/ml, and culturing the cells in suspension- or monolayer-cultures at about 37°C for 1-7 days, more preferably, 2-4 days, if necessary, while replacing the culture media with fresh ones. The cultures thus obtained usually contain the present polypeptides in a concentration of about 1-100  $\mu$ g/ml, which may vary depending on the types of the transformants or culture conditions used.

While the cultures thus obtained can be used intact as an IFN-y inducer, they can be usually subjected to the steps for purifying the present polypeptides before use, following the steps of separating the present polypeptides from the cells or the cell debris by filtration, centrifugation, etc., and, if necessary, which may follow a step for disrupting the cells by ultrasonication, cell-lytic enzymes, and/or detergents. To purify the present polypeptides, conventional techniques in this field for purifying biologically active polypeptides can be arbitrary used, e.g., salting-out, dialysis, filtration, concentration, fractional precipitation, ion-exchange chromatography, gel filtration chromatography, adsorption chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis and/or isoelectric focusing gel electrophoresis. The present polypeptides thus purified can be concentrated and/or lyophilized into liquids or solids depending on final uses. The monoclonal antibodies disclosed in Japanese Patent Application No.58,240/95 by the present applicant are extremely useful to purify the present polypeptides. Immunoaffinity chromatography using the antibodies can minimize the costs and the labors for obtaining the present polypeptides with a relatively high purity.

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The present polypeptides can be usually added to media for culturing immunocompetent cells to produce INF- $\gamma$ , or administered to humans to treat or prevent INF- $\gamma$  susceptive diseases. In the case of producing IFN- $\gamma$ , lymphocytes separated from mammalian peripheral bloods or established cell lines such as HBL-38 cells, Mo cells ATCC CRL8066, Jurkat cells ATCC CRL8163, HuT78 cells ATCC TIB161, EL4 cells ATCC TUB39, L12-R4 cells, and mutant strains thereof are suspended in culture media containing 0.1 ng - 1 µg/ml, preferably, 1 - 100 ng/ml of the present polypeptides. Then, the cells are cultured by conventional methods for about 1-100 hours, if necessary, in the presence of T-cell stimulating agents such as mitogens, interleukin 2, and anti-CD3 antibodies, and while replacing the culture media with fresh ones. To collect the IFN- $\gamma$  produced, the resulting cultures can be subjected to technique(s) appropriately selected from those conventional for purifying INF- $\gamma$ , e.g., salting-out, dialysis, filtration, concentration, fractional precipitation, gel filtration chromatography, ion-exchange chromatography, hydrophobic chromatography, adsorption chromatography, affinity chromatography, isoelectric focusing chromatography, gel electrophoresis, and isoelectric focusing gel electrophoresis, etc.

Since the present polypeptides induce production of IFN- $\gamma$ , agents for susceptive diseases containing the present polypeptides as an effective ingredient can induce production of IFN- $\gamma$  in human bodies when administered to human, and can treat and/or prevent IFN- $\gamma$ -susceptive diseases. When the present polypeptides have activities of enhancing cytotoxicities and/or inducing formation of killer cells such as NK cells, LAK cells (lymphokine-activated killer cells), and cytotoxic T cells, besides the IFN- $\gamma$  inducing activity, as in Examples of the present invention, described below, the killer cells are also involved in treating and/or preventing susceptive diseases. Thus, the wording "susceptive diseases" as referred to in the present invention includes all of the diseases which can be treated and/or prevented by the direct or indirect action of IFN- $\gamma$  and/or killer cells. The susceptive diseases are viral diseases including hepatitis, herpes, condyloma, and AIDS; infections including candidiasis, malaria, cryptococcoses, diseases caused by Yersinia infection, and tuberculosis; solid malignant tumors including renal carcinoma, mycosis fungoides, and chronic granulomatous diseases; blood-cell-derived malignant tumors including adult T cell leukemia, chronic myelogenous leukemia, and malignant lymphoma; immunopathies including allergies, rheumatism, and collagen diseases; and osteoporosis, etc. The present agents additionally containing interleukin 3 can completely treat or remit leukopenia and thrombopenia caused by radiation therapy or chemotherapy in treating malignant tumors, in addition to leukemia and myeloma.

Thus the present agents for susceptive diseases can be widely used for treating and/or preventing the aforesaid susceptive diseases in the forms of an antitumor agent, an antiviral agent, an antiseptic, an anti-immunopathic agent, a platelet-proliferating agent, and a leukocyte-proliferating agent, etc. The present agents can be usually processed into a liquid, paste, or solid form, containing 0.000001 - 100 w/w %, preferably, 0.0001 - 0.1 w/w % of the present polypeptides on a dry solid basis, while the form and the contents may vary depending on the uses and on the types and the symptoms of diseases to be treated and/or prevented.

The present agents can contain not only the present polypeptides solely but also other physiologically acceptable agents to form compositions, e.g., carriers, excipients, diluents, biological response modifiers and stabilizers, and if necessary, one or more other biologically active substances. The stabilizers can be proteins including serum albumins, and gelatins, saccharides including glucose, fructose, sucrose, maltose, lactose, trehalose, sorbitol, mannitol, maltitol, and lactitol, and buffers with phosphoric acid and/or succinic acid. Embodiments of the other biologically active substances are interferon-α, interferon-γ, interferon-γ, interleukin 2, interleukin 3, interleukin 12, TNF-α, TNF-β, granulo-

cyte-colony stimulating factor, granulocyte macrophage-colony stimulating factor, carboquone, cyclophosphamide, aclarubicin, thiotepa, busulfan, anbitabine, cytarabine, 5-fluorouracil, 5-fluoro-1-(tetrahydro-2-furyl)uracil, methotrexate, actinomycin D, chromomycin A3, daunorubicin, doxorubicin, bleomycin, mitomycin C, vincristine, vinblastine, L-asparaginase, radio gold colloidal, Krestin®, picibanil, lentinan, and Maruyama vaccine.

Among the above agents, those containing interleukin 2 are particularly useful because the interleukin 2 effects as a cofactor when the present polypeptides induce production of IFN- $\gamma$  by immunocompetent cells. Thus the agents, additionally containing a natural or recombinant interleukin 2, can induce production of IFN- $\gamma$  in a desired level by even immunocompetent cells that scarcely produce IFN- $\gamma$  by the single action of present polypeptides.

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The present agents additionally containing interleukin 12 can induce IFN-γ in a particularly high level which the present polypeptides or interleukin 12 per se cannot achieve. In addition, since the present polypeptides can enhance the inhibitory action of interleukin 12 on production of immunoglobulin E antibodies, the present agents with interleukin 12 are useful as an anti-immunopathic agent to treat and/or prevent atopic diseases such as atopic asthma, atopic bronchial asthma, hay fever, allergic rhinitis, atopic dermatitis, vascular edema, and atopic dyspepsia. Because there occasionally exists interleukin 12 in human bodies while in a relatively-low level, then the present polypeptides can achieve the desired effects in the human bodies alone.

The present agents include those in a unit of dose form, which means a physically separated and formed medicament suitable for administration, and contains the polypeptides required for a daily dose or in a dose from 1/40 to several folds (up to 4 folds) of the daily dose. Embodiments of such medicaments are injections, liquids, powders, granules, tablets, capsules, sublinguals, ophthalmic solutions, nasal drops and suppositories.

The present agents can be used for administering orally or parenterally to patients and for activating antitumor cells *in vitro* as described below, both of which effect to treat and/or prevent the susceptive diseases. For example, the present agents are usually administered orally to patients or parenterally to patients' intradermal tissues, subcutaneous tissues, muscles or veins as observing the patients' symptoms and recuperations at a dose in the range of about 0.1 - 50 mg/shot, preferably, one µg/shot to one mg/shot of the present polypeptides 1-4 times/day or 1-5 times/week for one day or one year.

The present agents can be also useful in so called "antitumor immunotherapies" using interleukin 2. The antitumor immunotherapies are generally classified into (i) a method administering the interleukin 2 directly to the bodies of patients with malignant tumors, and (ii) a method introducing antitumor cells activated by the interleukin 2 ex vivo to the patients (adoptive immunotherapy), any of which can exert significantly improved effects when used with the present polypeptides. For example, in the method (i), the present polypeptides can be administered to patients at an dose ranging from about 0.1 μg/shot/adult to one mg/shot/adult one to ten times simultaneously with or before the interleukin 2 administration. The dose of interleukin 2, which may vary depending on the types of the malignant tumors, the patients' symptoms and the dose of the present polypeptides, is usually in the range of 10,000 - 1,000,000 units/shot/adult. In the method (ii), to the media for culturing mononuclear cells or lymphocytes collected from patients with malignant tumors in the presence of the interleukin 2, the present polypeptides can be usually added with an amount of about 0.1 ng - 1 μg per 1×106 of the cell. After the cells are cultured for a prescribed period of time, NK cells or LAK cells are collected from the resulting cultures to be returned to the patients' bodies. Diseases as targets for the present antitumor immunotherapies are: solid malignant tumors such as colonic cancer, rectal cancer, gastric cancer, thyroid carcinoma, cancer of tongues, bladder carcinoma, choriocarcinoma, hepatoma, prostatic cancer, carcinoma uteri, laryngeal, lung cancer, breast cancer, malignant melanoma, Kaposi's sarcoma, cerebral tumor, neuroblastoma, tumor of ovaries, testicular tumor, osteosarcoma, cancer of pancreas, renal cancer, hypernephroma, and hemangioendothelioma; and blood cell malignant tumors such as leukemia and malignant lymphoma, etc.

The present DNAs, encoding the present polypeptides, are also useful in so called "gene therapies". According to conventional techniques in the gene therapies, the present DNAs can be introduced into patients with IFN-γ- and/or killer cell-susceptive diseases by direct injection after inserted into vectors derived from viruses such as retrovirus, adenovirus and adeno-associated virus, or after incorporated into cationic- or membrane fusible-liposomes. Alternatively, the present DNAs can be introduced into the patients by self-transplanting lymphocytes which have been collected from the patients before the DNAs have been introduced into. In adoptive immunotherapies with the gene therapies, the present DNAs can be introduced into effector cells similarly as using the conventional techniques. This can enhance cytotoxicities of the effector cells to tumor cells, resulting in improvement of the adoptive immunotherapy. In tumor vaccine therapy with the gene therapies, tumor cells from patients, into which the present DNAs can be introduced similarly as above, are self-transplanted after proliferated *ex vivo* up to give a desired cell number. The transplanted tumor cells act as vaccines in the patients to exert a improved antitumor immunity specific to the antigens. Thus the present DNAs exhibit remarkable effects in the gene therapies for diseases including viral diseases, microbial diseases, malignant tumors, and immunopathies. General procedures for the gene therapies are detailed in Takashi SHIMADA, Izumi SAITO and Keiya OZAWA eds., *Jikken-Igaku-Bessatsu*, *Biomanual UP Series*, *Idenshichiryo-no-Kisogijutsu* (Basic techniques for the gene therapy), (Tokyo, Japan: Yodosha Co., Ltd., 1996).

The following examples explain the present invention: Examples A-1 to A-9 describe preferred embodiments of

the polypeptides and the process for producing thereof according to the present invention, and Examples B-1 to B-5 describe the preferred embodiments of the agents for susceptive diseases according to the present invention. The techniques in Examples A-1 to A-9 are conventional ones used in this field, which are detailed in publications, e.g., Toshio KUROKI, Masaru TANIGUCHI and Mitsuo OSHIMURA eds., *Jikken-Igaku-Bessatsu*, *Saibo-Kogaku Handbook* (The handbook for the cell engineering), (Tokyo, Japan: Yodosha. Co., Ltd., 1992), and Takashi YOKOTA and Kenichi ARAI eds., *Jikken-Igaku-Bessatsu*, *Biomanual Series 3*, *Idenshi-Cloning-Jikken-Ho* (The experimental methods for the gene cloning), (Tokyo, Japan: Yodosha Co., Ltd., 1993).

Example A-1

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Production of polypeptide

Example A-1(a)

### 15 Construction of recombinant DNA

Genomic DNA was collected by conventional manner from BALL-1 cells, ATCC RCB0256, an established cell line derived from human acute lymphocytic leukemia, and oligonucleotides with the nucleotide sequences of 5'-ACACCTC-GAGCCACCATGGCCTTGACCTTTGCTTTAAC-3' as a sense primer (the sense primer 1) and of 5'-TTGCCAAAG-TAGCCCACAGAGCAGCTTG-3' as an antisense primer (the antisense primer 1) were chemically synthesized based on the nucleotide sequence for the signal peptide of the subtype  $\alpha$ 2b of human interferon- $\alpha$ , shown in SEQ ID NO:24, described in K. Henco et al. *Journal of Molecular Biology*, Vol.185, pp.227-260 (1985). In a 0.5 ml-reaction tube, one  $\mu$ g of the genomic DNA,  $10\mu$ l of  $10 \times$  PCR buffer, one  $\mu$ l of 25 mM dNTP mix, and adequate amounts of the sense primer 1 and the antisense primer 1 were mixed, and sterilized distilled water was added to the mixture to give a volume of 99  $\mu$ l. To the mixture, one  $\mu$ l of 2.5 units/ $\mu$ l Pfu DNA polymerase was further added. The mixture was subjected to 30 cycles of incubations at 94°C, 600C, and 72°C for one minute, respectively, to perform a PCR, resulting in obtaining a DNA fragment (the DNA fragment 1) which comprised the nucleotide sequence of SEQ ID NO:24, a site recognized by a restriction enzyme of *Xho*l, linked to the 5'-terminus of the SEQ ID NO:24, and a sequence of 1st-11th nucleotides in SEQ ID NO:25, linked to the 3'-terminus of the SEQ ID NO:24.

The recombinant DNA "pHIGIF", containing the nucleotide sequence of SEQ ID NO:25 encoding the wild-type polypeptide with the amino acid sequence of SEQ ID NO:4, was prepared according to the methods described in Japanese Patent Kokai No.193,098/96 by the present applicant. The wild type polypeptide, with the amino acid sequence of SEQ ID NO:4, contains partial amino acid sequences of SEQ ID NOs:1-3 in the regions of 16th-21st, 30th-35th, and 51st-55th amino acids. Oligonucleotides with the nucleotide sequences of 5'-CTGCTCTGTGGGCTACTTT-GGCAAGCTTGAATC-3' as a sense primer (the sense primer 2) and 5'-ACACGCGGCCGCCTAGTCTTCGTTTT-GAACAG-3' as an antisense primer (the antisense primer 2) were chemically synthesized in usual manner based on SEQ ID NOs:25 and 26. In a 0.5 ml-reaction tube, one ng of the recombinant DNA "pHIGIF", 10µl of 10 × PCR buffer, one µl of 25 mM dNTP mix and adequate amounts of the sense primer 2 and the antisense primer 2 were mixed, and then sterilized distilled water was added to the mixture to give a volume of 99µl. To the mixture, one µl of 2.5 units/µl Pfu DNA polymerase was further added. The mixture was subjected to 30 cycles of incubations at 94°C, 60°C and 72°C for one minute, respectively, to perform a PCR, resulting in obtaining a DNA fragment (the DNA fragment 2) which comprised the nucleotide sequence of SEQ ID NO:25, a termination codon of 5'-TAG-3' and a site recognized by a restriction enzyme of *Not*1, linked to the 5'-terminus of the SEQ ID NO:25, and a sequence of 57th-69th nucleotides in SEQ ID NO:24, linked to the 3'-terminus of the SEQ ID NO:25.

In a 0.5 ml-reaction tube, one ng of the DNA fragments 1 and 2 each,  $10\,\mu$ l of  $10\,\times$  PCR buffer, and one  $\mu$ l of 25 mM dNTP mix were mixed, and sterilized distilled water was added to the mixture to give a volume of  $99\mu$ l. The mixture was incubated at  $94^{\circ}$ C for 3 minutes and slowly cooled to  $37^{\circ}$ C, and incubated at the temperature for 15 minutes. The mixture was given one  $\mu$ l of 2.5 units/ $\mu$ l Pfu DNA polymerase and slowly heated to  $72^{\circ}$ C, and then incubated at the temperature for 2 minutes. After added adequate amounts of the sense primer 1 and the antisense primer 2, the mixture was subjected to 30 cycles of incubations at 940C for one minute, at  $60^{\circ}$ C for one minute, and at  $72^{\circ}$ C for 30 seconds, to perform a PCR, resulting in obtaining a DNA fragment (the DNA fragment 3) which comprised the nucleotide sequence of SEQ ID NO:26.

An oligonucleotide with the nucleotide sequence of 5'-CTCTGTGAGAAAATTTCAACTC-3', as a mutagenic sense primer to replace the 283rd nucleotide of guanine in SEQ ID NO:26 with a cytosine, was chemically synthesized by usual manner. A PCR was performed similarly as that for obtaining the DNA fragment 1, but using the DNA fragment 3 as a template and the mutagenic sense primer for the sense primer 1. The PCR resulted in obtaining a DNA fragment (the DNA fragment 4) which comprised a nucleotides sequence identical to 276th-570th nucleotides in SEQ ID NO:26 except for the 287th nucleotide replaced with a cytosine.

An oligonucleotide with the nucleotide sequence of 5'-GAGTTGAAATTTTCTCAGACTTCACAGAG-3', as a mutagenic antisense primer to replace the 287th nucleotide of guanine in SEQ ID NO:26 with a cytosine, was chemically synthesized by usual manner. A PCR was performed similarly as that for obtaining the DNA fragment 2, but using the DNA fragment 3 as a template and the mutagenic antisense primer for the antisense primer 1. The PCR resulted in obtaining a DNA fragment (the DNA fragment 5) which comprised a nucleotides sequence identical to Ist-304th nucleotides in SEQ ID NO:26 except for the 287th nucleotide replaced with a cytosine.

A PCR was performed similarly as that for obtaining the DNA fragment 3, but using the DNA fragments 4 and 5 as templates, to obtain a DNA fragment (the DNA fragment 6) containing a nucleotide sequence encoding the amino acid sequence of SEQ ID NO:6. The DNA fragment 6 comprised the nucleotide sequence of SEQ ID NO:15, the nucleotide sequence of SEQ ID NO:24 and a site recognized by a restriction enzyme *Xho*I, linked to the 5'-terminus of the SEQ ID NO:15, and a termination codon of the nucleotides of 5'-TAG-3' and a site recognized by a restriction enzyme *NoI*I, linked to 3'-terminus of the SEQ ID NO:15.

After the DNA fragment 6 by restriction enzymes Xhol and Notl was cleaved to generate a DNA fragment of 555 bps, 25 ng of the generated DNA fragment was mixed with 10 ng of a plasmid vector "pCDM8", commercialized by Invitrogen Corporation, San Diego, USA, which had been cleaved by the Xhol and Notl, and then the mixture was incubated at 16°C for 30 minutes using a ligation kit "LIGATION KIT VERSION 2", commercialized by Takara Shuzo Co., Tokyo, Japan. By cloning, an autonomously replicable recombinant DNA "pCSHIGIF/MUT12" consisting of 4,494bp was obtained. As shown in FIG. 1, in the recombinant DNA "pCSHIGIF/MUT12", a cDNA "IGIF/MUT12" with the nucleotide sequence of SEQ ID NO:15 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype  $\alpha$ 2b of human interferon- $\alpha$ . As shown in the accompanied amino acid sequence, the nucleotide sequence of SEQ ID NO:15 encodes the amino acid sequence of SEQ ID NO:6, derived from the wild-type polypeptide with SEQ ID NO:4 by replacing the cysteine at the 68th position.

For a control, an autonomously replicable recombinant DNA "pCSHIGIF/WT" was prepared similarly as above excepting the DNA fragment 6 replaced with the DNA fragment 3. As shown in FIG. 2, in the recombinant DNA "pC-SHIGIF/WT", a cDNA "IGIF/WT" with the nucleotide sequence of SEQ ID NO:25, encoding the wild-type polypeptide, was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of subtype α2b of human interferon-α.

#### Example A-1(b)

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### Production of polypeptide by transformant

The recombinant DNA "pCSHIGIF/MUT12", obtained in Example A-1(a), was introduced by conventional competent-cell method into an *Escherichia coli* strain "MC1061/P3", commercialized by Invitrogen Corporation, San Diego, USA, to obtain a transformant. The transformant was cultured in L medium (pH 7.2) containing 20 μg/ml ampicillin and 10 μg/ml tetracycline at 37°C for 18 hours under shaking conditions. The resulting culture was centrifuged to separate the cells, and the separated cells were subjected to conventional alkali-SDS method to extract the recombinant DNA.

2.5 ml of DME medium (pH 7.4) supplemented with 10 v/v % fetal bovine serum was put into each well of six-well microplates, and 1.8×10<sup>5</sup> cells of COS-1, ATCC CRL1650, an established cell line derived from African green monkey kidney, was inoculated into each well. The microplates were incubated at 370C for 24 hours in a 5 v/v % CO2 incubator. After the incubation, the media were removed, and the wells were washed with DME medium containing 50 mM Tris-HCl buffer (pH 7.4). To each well, 1.8 ml of DME medium containing 2.8 µg/ml of the recombinant DNA obtained above, 50 mM Tris-HCl buffer (pH 7.4), 0.4 mg/ml DEAE-dextran and 0.1 mM chloroquine was added, and the microplates were incubated at 37°C for 4 hours in a 5 v/v % CO<sub>2</sub> incubator. After the incubation, the media were removed, and 2.5 ml of 10 mM phosphate buffer (pH 7.4) containing 10 v/v % dimethylsulfoxide and 140 mM NaCl was added to each well, and then the microplates were stood at ambient temperature for 2 minutes. After the standing, the buffers were removed, and the wells were washed with DME medium containing 50 mM Tris-HCl buffer (pH 7.4). To each well, 2.5 ml of a culture medium "COS MEDIUM", commercialized by COSMOBIO Co., Ltd., Tokyo, Japan, was added, and the microplates were incubated at 37°C for 3 days in a 5 v/v % CO2 incubator to culture the cells. The resulting culture was analyzed by Western blotting using the monoclonal antibody described in Japanese Patent Kokai No.231,598/96. The analysis proved that the present polypeptide, capable of inducing production of IFN-γ by immunocompetent cells and containing an amino acid sequence derived from SEQ ID NO:4 by replacing the cysteine at 68th position with a serine, was produced in the culture in an amount of about 20 ng/ml.

As a control experiment, the recombinant DNA "pCSHIGIF/WT" obtained in Experiment A-1(a) was treated similarly as the recombinant DNA "pCSHIGIF/MUT12". Consequently, the wild-type polypeptide capable of inducing production of IFN-7 was produced in the culture in an amount of about one ng/ml. This yield was no more than 5% of that obtained by using the recombinant DNA "pCDHIGIF/MUT12". This indicates that the polypeptide according to the present invention, in this Example, is more stable and exhibits biological activities higher than the wild-type polypeptide.

### Example A-1(c)

#### Purification of polypeptide

The culture containing the present polypeptide that was obtained in Experiment A-1(b) was centrifuged to collect a supernatant. After the supernatant was fed to a column, which was packed with a gel for immunoaffinity chromatography using the monoclonal antibody, prepared according to the methods disclosed in Japanese Patent Kokai No. 231,598/96 by the present applicant, and preliminarily washed with phosphate-buffered saline (hereinafter abbreviated as "PBS"), a fresh PBS was run through the column to wash, and then 0.1 M glycine-HCl buffer (pH 2.5) containing one M NaCl was run to elute. From the eluted fractions, those containing the polypeptide capable of inducing production IFN-γ by immunocompetent cells were collected. The collected fractions were dialyzed against PBS at 40C for 18 hours, and then concentrated by membrane-filtration followed by lyophilization to obtain a solid polypeptide with a purity of about 95 % or higher and a recovery of about 50 % to the culture of the starting material. In parallel, the culture containing the wild-type polypeptide, obtained by using the recombinant DNA "pCSHIGIF/WT", was purified similarly as above for a control in analyzing the physicochemical properties as described below.

### Example A-1(d)

#### Molecular weight

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SDS-Polyacrylamide gel electrophoresis of the polypeptide in Example A-1(c) in the presence of 2 w/v % dithiothreitol as a reducing agent, according to the method described in U. K. Laemli, *Nature*, Vol.227, pp.680-685 (1970), exhibited a main band of a protein capable of inducing IFN- $\gamma$  at a position corresponding to a molecular weight of about 18,000-19,500 daltons. The molecular weight makers used were bovine serum albumin (67,000 daltons), ovalbumin (45,000 daltons), carbonic anhydrase (30,000 daltons), soy bean trypsin inhibitor (20,100 daltons), and  $\alpha$ -lactoalbumin (14,000 daltons). Example A-1(e)

### N-Terminal amino acid sequence

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Conventional analysis using a protein sequencer "MODEL 473A", commercialized by Perkin-Elmer Corp., Norwalk, USA, revealed that the polypeptide in Example A-1(c) had the amino acid sequence of SEQ ID NO:27 in the N-terminal region. Example A-1(f)

### Stability

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The present polypeptide or the wild-type polypeptide, in Example A-1(c), was dissolved in a culture medium "COS MEDIUM", commercialized by COSMOBIO Co., Ltd., Tokyo, Japan, to give about 10 ng/ml, and the solution was incubated at 40°C for 24 hours. After 0, 0.5, 1, 2, 4, 6, 8, 12, or 24 hours from starting the incubation, a portion of each solution was sampled. The samples were individually assayed on IFN-γ inducing activity, according to the methods described below, in Example A-1(g), to study the time course of the activity upon the incubation. Percentage (%) of the residual activity at every point was calculated based on the activity at the starting point. The results are in FIG. 3.

As shown in FIG. 3, the polypeptide in this Example was more stable and retained the activity longer than the wildtype polypeptide. This evidences that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

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### Example A-1(q)

### Production of IFN-γ by immunocompetent cells

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KG-1 cells, ATCC CCL246, an established cell line derived from human acute myelogenous leukemia, were inoculated into RPMI-1640 medium (pH 7.4) with no sera to give a density of 3×10<sup>5</sup> cells/ml and cultured at 37°C for 4 days in a 5 v/v % CO<sub>2</sub> incubator. The cultured cells were collected and suspended to give a density of 3×10<sup>6</sup> cells/ml in RPMI-1640 medium (pH 7.4) supplemented with 10 v/v % fetal bovine serum. 0.1 ml of the cell suspension was put into each well of 96-well microplates, and 0.1 ml of a solution containing the present polypeptide or the wild-type polypeptide, obtained in Example A-1(c), which had been diluted appropriately, was added to each well. Thereafter, the cells were cultured at 37°C for 24 hours in a 10 v/v % CO<sub>2</sub> incubator. 0.1 ml of supernatants of the cultures were collected from the wells and examined on productions of IFN-γ by conventional enzyme-immunoassay. As a blank, an experiment was taken in parallel identically as above but using no polypeptides. Table 1 shows the results. The pro-

ductions of IFN-γ in Table 1 were expressed with international units (IU), calculated based on the IFN-γ standard Gg23-901-530, obtained from the International Institute of Health, USA.

Table 1

Polypeptide concentration, ng/ml	Production of IFN-γ, IU/ml*
0	0 (0)
0.1	0.7 (0.6)
0.2	3.0 ( 2.4)
0.4	8.1 ( 7.4)
0.8	20.0 (18.9)
1.0	30.0 (25.9)

<sup>\*)</sup> Value in parentheses represents the production of IFN-y when using the wild-type polypeptide.

Table 1 indicates that the present polypeptide acted on KG-1, an immunocompetent cell, to induce the production of IFN-γ. The IFN-γ production was equal to or higher than that induced by the wild-type polypeptide.

### Example A-1(h)

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#### Enhancement of cytotoxicity of NK cells

A fresh blood was collected from a healthy donor by using a syringe containing heparin, and the blood was diluted with the equal volume of PBS. The diluted blood was overlaid on FICOLL and centrifuged to obtain high-density lymphocytes. The lymphocytes were suspended to give a density of 1 × 10<sup>6</sup> cells/ml in RPMI-1640 medium (pH 7.2) containing 10 μg/ml kanamycin, 5 × 10<sup>-5</sup> M 2-mercaptoethanol and 10 v/v % fetal bovine serum. 0.5 ml of the cell suspension was put into each well of 12-well microplates. To each well, the present polypeptide or the wild-type polypeptide, obtained in Example A-1(c), in 1.5 ml solution was added after appropriately diluted with a fresh preparation of the medium, and 0.5 ml of a fresh preparation of the medium with or without 50 units/ml of a recombinant human interleukin 2 was further added. Thereafter, the cells were cultured at 37°C for 24 hours in a 5 v/v % CO<sub>2</sub> incubator. The cultured cells were washed with PBS to obtain cultured lymphocytes containing NK cells as effector cells.

K-562 cells, ATCC CCL243, an established cell line derived from human chronic myelogenous leukemia, as target cells sensitive to NK cells, were labelled with  $^{51}$ Cr by a conventional method, and  $1\times10^4$  cells of the labelled cells were put into each well of 96-well microplates. To the wells, the cultured lymphocytes obtained above were added to give the ratios of 2.5:1, 5:1 and 10:1 between the effector and the target cells, before cultured at 37°C for 4 hours in a 5 v/ v %  $CO_2$  incubator. Thereafter, the culture supernatants were examined on the radioactivity by conventional manner to estimate the number of killed cells. Percentage (%) of the killed cells to the target cells tested in each system was calculated to evaluate the cytotoxicity. Table 2 shows the results.

### Example A-1(i)

### Induction of LAK cell formation

Cultured lymphocytes containing LAK cells as effector cells were prepared by a procedure similar as in Example A-1(g) excepting the culturing time replaced with 72 hours. Raji cells, ATCC CCL86, an established cell line derived from human Burkitt lymphoma, as target cells non-sensitive to NK cells, was labelled with <sup>51</sup>Cr according to the conventional method. 1 × 10<sup>4</sup> of the labelled cells were put into each well of 96-well microplates, and the cultured lymphocytes were added to the wells to give the ratios of 5:1, 10:1, and 20:1 between the effector and the target cells, before cultured at 37°C for 4 hours in a 5 v/v % CO<sub>2</sub> incubator. Thereafter, similarly as in Example A-1(g), the culture supernatants were examined on the radioactivity to evaluate the cytotoxicity. Table 3 shows the results.

5 10 15		Cytotoxicity, %**	[Effector Cells] : [Target Cells] 2.5:1 5:1	2 (22) 35 (35) 65 (6 0 (30) 48 (48) 73 (7	5 (23) 41 (36) 65 (6	1 (32) 54 (50) 69 (7	28 (25) 49 (39) 66 (68) 36 (35) 58 (52) 79 (78)	0 (29) 53 (47) 77 (7	2 (41) 62 (59) 82 (8	3 (37) 56 (50) 84 (8	7 (52) 78 (70) 96 (9	Value in parentheses represents
25	Table 2		unit/ml									12 M. **; Valuthe wild-type
30		11	2,	00	0	0.0	. 10	0	10	0	0.	of 10-
35			Concentration Interleukin 1	_		7	М		-		1	a molarity bited when
40 .		) t	b, pM*									
45		40 00000	Concentation of Polypeptide, pM*	00	0.5	0.5	വ വ	20	20	200	200	NOTE) *: "pM" means the cytotoxicity exhi
50		C	3 <b>G</b>									NOTE)

As shown in Table 2, the present polypeptide enhanced the cytotoxicity of NK cells, and the enhancement was equal to or higher than that of the wild-type polypeptide. The enhancement was augmented by the co-existing of interleukin 2.

5 10 15		Cytotoxicity, %**	<pre>[Effector Cells] : [Target Cells] 5:1 10:1 20:1</pre>	1 (11) 21 (21) 34 (	5 (15) 28 (28) 38 (	4 (13) 24 (22) 34 (	8 (17) 32 (31) 42 (	21 (19) 36 (34) 50 (48)	2 (20) 41 (25) 49 (	6 (23) 52 (42) 56 (	7 (27) 44 (34) 61 (	3 (31) 59 (54) 72 (	Value in parentheses represents type polypeptide.
25	Table 3		unit/ml								•		<sup>12</sup> Μ. **: Valι the wild-type
30		# C 20 F + C 2 + 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		0	10	0	10	10	0	10	0	10	of 10 <sup>-3</sup> using
35			Interleukin										a molarity ibited when
45		+ + + + + + + + + + + + + + + + + + + +	Polypeptide, pM*	0	0	0.5	. s	വാ	50	50	500	200	NOTE) *: "pM" means the cytotoxicity exhi
													NO th

As shown in Table 3, the present polypeptide induced the formation of LAK cells, and the induction was equal to or higher than that of the wild-type polypeptide. The induction was augmented by the co-existing of interleukin 2.

### Example A-1(i)

#### Acute toxicity test

The present polypeptide in Example A-1(c) was percutaneously, perorally or intraperitoneally administered to 8-week-old mice in usual manner. As a result, the LD<sub>50</sub> of the present polypeptide proved to be about one mg or higher per one kg of the body weight, independently of the administration routs. This evidences that the present polypeptide can be incorporated into pharmaceuticals for humans without anxiety.

#### Example A-2

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### Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT21" containing the nucleotide sequence of SEQ ID NO:16 was obtained by a procedure similar as in Example A-1(a) but using the DNA fragment 6, obtained in Example A-1(a), as a template, and an oligonucleotide with the nucleotide sequence of 5'-CTGATTCTGACTCTAGATAATGC-3' and an oligonucleotide with the nucleotide sequence of 5'-GCATTATCTCTAGAGTCAGAATCAG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 38th position in SEQ ID NO:4 with a serine. As shown in FIG. 4, in the recombinant DNA "pCSHIGIF/MUT21", a cDNA "IGIF/MUT21" encoding the amino acid sequence of SEQ ID NO:7 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:7 in an amount of about 50 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

### Example A-3

### Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT25" containing the nucleotide sequence of SEQ ID NO:17 was obtained by a procedure similar as in Example A-1(a) but using the DNA fragment 6, obtained in Example A-1(a), as a template, and an oligonucleotide with the nucleotide sequence of 5'-CTTTCTAGCTTCTGAAAAAGAGAGAGAG-3' and an oligonucleotide with the nucleotide sequence of 5'-CTCTCTCTTTTTCAGAAGCTAGAAAG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 127th position in SEQ ID NO:4 with a serine. As shown in FIG. 5, in the recombinant DNA "pCSHIGIF/MUT25", a cDNA "IGIF/MUT25" encoding the amino acid sequence of SEQ ID NO:8 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype a2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:4 in an amount of about 30 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

#### Example A-4

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### Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT32" containing the nucleotide sequence of SEQ

ID NO:18 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT21" as a template, encoding the amino acid sequence of SEQ ID NO:7, in the recombinant DNA "pCSHIGIF/MUT21" obtained in Example A-2, and an oligonucleotide with the nucleotide sequence of 5'-CTTTCTAGCTTCTGAAAAAGAGAGAG-3' and an oligonucleotide with the nucleotide sequence of 5'-CTCTCTTTTTCAGAAGCTAGAAAG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 127th position in SEQ ID NO:4 with a serine. As shown in FIG. 6, in the recombinant DNA "pCSHIGIF/MUT32", a cDNA "IGIF/MUT32" encoding the amino acid sequence of SEQ ID NO:9 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype a2b of human interferon-a.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:9 in an amount of about 80 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

### Example A-5

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### Production of polypeptide

An autonomously replicable recombinant DNA "pCSHIGIF/MUT41" containing the nucleotide sequence of SEQ ID NO:19 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT32" as a template, with the nucleotide sequence of SEQ ID NO:18, in the recombinant DNA "pCSHIGIF/MUT32" obtained in Example A-4, and an oligonucleotide with the nucleotide sequence of 5'-CAACTCTCTCTCTGAGAACAA-3' and an oligonucleotide with the nucleotide sequence of 5'-TTGTTCTCAGAGGAGAGAGTTG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 76th position in SEQ ID NO:4 with a serine. As shown in FIG. 7, in the recombinant DNA "pCSHIGIF/MUT41", a cDNA "IGIF/MUT41" encoding the amino acid sequence of SEQ ID NO:10 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype a2b of human interferon-a.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:10 in an amount of about 6 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

### Example A-6

#### Production of polypeptide

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An autonomously replicable recombinant DNA "pCSHIGIF/MUT35" containing the nucleotide sequence of SEQ ID NO:20 was obtained by a procedure similar as in Example A-1(a) but using the cDNA "IGIF/MUT21" as a template, encoding the amino acid sequence of SEQ ID NO:7, in the recombinant DNA "pCSHIGIF/MUT21" obtained in Example A-2, and an oligonucleotide with the nucleotide sequence of 5'-CTCTCCGCTGAGAACAAATTATTTCC-3' and an oligonucleotide with the nucleotide sequence of 5'-TTTGTTCTCAGCGGAGAGAGTTG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 76th position in SEQ ID NO:4 with an alanine. As shown in FIG. 8, in the recombinant DNA "pCSHIGIF/MUT41", a cDNA "IGIF/MUT35" encoding the amino acid sequence of SEQ ID NO:11 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

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The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:11 in an amount of about 60 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in

the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

### Example A-7

### Production of polypeptide

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An autonomously replicable recombinant DNA "pCSHIGIF/MUT42" containing the nucleotide sequence of SEQ ID NO:21 was obtained by a procedure similar as in Example A-1 (a) but using the cDNA "IGIF/MUT32" as a template, encoding the amino acid sequence of SEQ ID NO:18, in the recombinant DNA "pCSHIGIF/MUT32" obtained in Example A-4, and an oligonucleotide with the nucleotide sequence of 5'-CTCTCCGCTGAGAACAAATTATTTCC-3' and an oligonucleotide with the nucleotide sequence of 5'-TTTGTTCTCAGCGGAGAGAGTTG-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 76th position in SEQ ID NO:4 with an alanine. As shown in FIG. 9, in the recombinant DNA "pCSHIGIF/MUT42", a cDNA "IGIF/MUT42" encoding the amino acid sequence of SEQ ID NO:12 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

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The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:12 in an amount of about 30 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-1. As a result, the polypeptide in this Example proved to be similar to that in Example A-1 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 3, the results of the analysis on stability, obtained according to the method in Example A-1(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

#### 30 Example A-8

### Production of polypeptide

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A PCR was performed similarly as the PCR for obtaining the DNA fragment 1 in Example A-1(a) but using an oligonucleotide with the nucleotide sequence of 5'-CGGCCAAAGTTGCCCACAGAGCAGCTTG-3', chemically synthesized, for the antisense primer 1. The PCR resulted in obtaining a DNA fragment (the DNA fragment 7) which comprised the nucleotide sequence of SEQ ID NO:24, a site recognized by a restriction enzyme *Xho*I, linked to the 5'-terminus of the SEQ ID NO:24, and a sequence of Ist-Ilth nucleotides in the nucleotide sequence of SEQ ID NO:28, linked to the 3'-terminus of the SEQ ID NO:24.

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The recombinant DNA "pMGTG-1", containing the nucleotide sequence of SEQ ID NO:28 encoding the wild-type polypeptide with the amino acid sequence of SEQ ID NO:5, was prepared according to the methods described in Japanese Patent Kokai No.27,189/96 by the present applicant. The wild type polypeptide, with the amino acid sequence of SEQ ID NO:5, contains partial amino acid sequences of SEQ ID NOs:1, 2 and 3 in the parts consisting of 16th-21st, 29th-34th, and 50th-54th amino acids, respectively. Oligonucleotides with the nucleotide sequence of 5'-CTGCTCT-GTGGGCAACTTTGGCCGACTTCACTG-3' as a sense primer (the sense primer 3) and 5 '-ACACGCG-GCCGCCTAACTTTGATGTAAGTTAG-3' as an antisense primer (the antisense primer 3) were chemically synthesized. Thereafter, a PCR was performed similarly as that for obtaining the DNA fragment 2 in Example A-1(a) but using the recombinant DNA "pMGTG-1"; the sense primer 3 and the antisense primer 3 for the recombinant DNA "pHIGIF", the sense primer 2 and the antisense primer 2, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 8) which comprised the nucleotide sequence of SEQ ID NO:28, a termination codon of 5'-TAG-3' and a site recognized by a restriction enzyme *Not*1, linked to the 3'-terminus of the SEQ ID NO:28, and a sequence of 57th-69th nucleotides in the nucleotide sequence of SEQ ID NO:24, linked to the 5'-terminus of the SEQ ID NO:28.

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A PCR was performed similarly as that for obtaining the DNA fragment 3 in Example A-1(a) but using the DNA fragments 7 and 8 and the antisense primer 3, obtained above, for the DNA fragments 1 and 2 and the antisense primer 2, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 9) comprising the nucleotide sequence of SEQ ID NO:29.

A PCR was performed similarly as that for obtaining the DNA fragment 4 in Example A-1(a) but using the DNA fragment 9 for, the DNA fragment 3, the antisense primer 3 for the antisense primer 2, and an oligonucleotide with the

nucleotide sequence of 5'-GGCCGACTTCACGCTACAACC-3' for the mutagenic sense primer, to replace 103rd and 104th nucleotides of thymine and guanine in SEQ ID NO:29 with a guanine and cytosine, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 10) comprising a nucleotide sequence identical to 91st-570th nucleotides in SEQ ID NO:29 except for the 103rd and 104th replaced with a guanine and a cytosine, respectively.

A PCR was performed similarly as that for obtaining the DNA fragment 5 in Example A-1(a) but using the DNA fragment 9 for the DNA fragment 3, and an oligonucleotide with the nucleotide sequence of 5'-GGTTGTAGCGT-GAAGTCGGCC-3' for the mutagenic antisense primer, to replace 103rd and 104th nucleotides of thymine and guanine in SEQ ID NO:29 with a guanine and cytosine, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 11) comprising a nucleotide sequence identical to 1st-111th nucleotides in SEQ ID NO:29 except for the 103rd and 104th, replaced with a quanine and cytosine, respectively.

A PCR was performed similarly as that for obtaining the DNA fragment 3 in Example A-1(a) but using the DNA fragments 10 and 11 and the antisense primer 3, obtained above, for the DNA fragments 1 and 2 and the antisense primer, respectively. The PCR resulted in obtaining a DNA fragment (the DNA fragment 12) comprising the nucleotide sequence of SEQ ID NO:24 and a site recognized by a restriction enzyme *Xho*I, linked to the 5'-terminus of the SEQ ID NO:22, and a termination codon of 5'-TAG-3' and a site recognized by a restriction enzyme *Not*I, linked to the 3'-terminus of the SEQ ID NO:22.

The DNA fragment 12 was treated similarly as the DNA fragment 6, according the procedure for obtaining the recombinant DNA "pCSHIGIF/MUT12" in Example A-1(a), to obtain a autonomously replicable recombinant DNA "pCSMIGIF/MUT11". As shown in FIG. 10, in the recombinant DNA "pCSMIGIF/MUT11", a cDNA "mIGIF/MUT11" with the nucleotide sequence of SEQ ID NO:22 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype  $\alpha$ 2b of human interferon- $\alpha$ . As shown in the accompanied amino acid sequence, the SEQ ID NO:22 encodes an amino acid sequence derived from the wild type polypeptide with SEQ ID NO:5 by replacing the cysteine at the 7th position with an alanine.

For a control, an autonomously replicable recombinant DNA \*pCSMIGIF/WT\* was prepared similarly as the procedure for obtaining the recombinant DNA \*pCSHIGIF/MUT12\* but treating the DNA fragment 9 for the DNA fragment 6. As shown in FIG. 11, in the recombinant DNA \*pCSMIGIF/WT\*, a cDNA \*mIGIF/WT\* with the nucleotide sequence of SEQ ID NO:28, encoding the wild-type polypeptide, was linked to downstream of the nucleotide sequence "IFNss\*, encoding the signal peptide of subtype a2b of human interferon-α.

#### Example A-8(b)

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### Production of polypeptide by transformant

According to the procedure for the production of the polypeptide in Example A-1(b) but using the recombinant DNA "pCSMIGIF/MUT12" for "pCSHIGIF/MUT11", the recombinant DNA was extracted, the DNA was introduced into COS-1 cells, and the COS-1 cells with the DNA was cultured to obtain a culture. The culture was analyzed by Western blotting using the monoclonal antibody described in Japanese Patent Kokai No.217,798/96 by the present applicant. The analysis proved that the present polypeptide capable of inducing production of IFN- $\gamma$  by immunocompetent cells, with the amino acid sequence derived from SEQ ID NO:5 by replacing the cysteine at 7th position with an alanine, was produced in the culture in an amount of about 20 ng/ml.

As a control, treating the recombinant DNA "pCSHMIGIF/WT" similarly as above produced the wild-type polypeptide capable of inducing production of IFN- $\gamma$  by immunocompetent cells. The production of the wild-type polypeptide was significantly lower than that obtained by using "pCSMIGIF/MUT11", descrived above. This evidences that the present polypeptide in this Example is more stable and exhibits the biological activities higher than the wild-type polypeptide.

#### Example A-8(c)

### Purification of polypeptide

The culture containing the present polypeptide, in Example A-8(b), was centrifuged to collect a supernatant. The supernatant was fed to a column packed with a gel for immunoaffinity chromatography using the monoclonal antibody, prepared by the method described in Japanese Patent Kokai No.217,798/96 by the present applicant, and preliminarily washed with PBS. After a fresh PBS was run through the column to wash, 35 mM ethylamine (pH 10.8) was run to elute. From the eluted fractions, those containing the polypeptide capable of inducing production IFN- $\gamma$  by immunocompetent cells were collected. The collected fractions were dialyzed against PBS at 4°C for 18 hours, and then concentrated by membrane-filtration followed by lyophilization to obtain a solid polypeptide with a purity of about 95%. In parallel, the culture containing the wild-type polypeptide, obtained by using the recombinant DNA \*pCSMIGIF/

WT", was purified similarly as above for a control in analyzing the physicochemical properties as described below.

#### Example A-8(d)

### 5 Molecular weight

SDS-Polyacrylamide gel electrophoresis of the present polypeptide in Example A-8(c), similarly as in Example A-1(d), exhibited a main band of polypeptide capable of inducing production at a position corresponding to a molecular weight of about 18,500-19,500 daltons.

#### Example A-8(e)

### N-Terminal amino acid sequence

By analyzing similarly as in Example A-1(e), the present polypeptide in Example A-8(c) was proved to contain the amino acid sequence of SEQ ID NO:30 in the N-terminus.

#### Example A-8(f)

#### 20 Stability

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The present polypeptide or the wild-type polypeptide, in Example A-8(c), was dissolved in PBS containing 0.2 g/ml maltose, and the solution was incubated at 40°C for 24 hours. After 0, 3, 9, or 24 hours from starting the incubation, a portion of each solution was sampled. The samples were individually assayed on IFN-γ inducing activity, according to the methods described below, in Example A-8(g), to study the time course of the activity upon the incubation. Percentage (%) of the residual activity at every point was calculated based on the activity at the starting point. The results are in FIG. 12.

As shown in FIG. 12, the present polypeptide in this Example was more stable and retained the activity longer than the wild-type polypeptide. This evidences that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

### Example A-8(g)

### Production of IFN-γ by immunocompetent cells

Splenocytes were collected from C3H/HeJ mice as immunocompetent cells. The splenocytes were suspended in RPMI-1640 medium supplemented with 10 v/v % fetal bovine serum. The suspensions were given the present polypeptide or the wild-type polypeptide, in Example A-8(a), in the presence or absence of concanavalin A or interleukin 2. Thereafter, the splenocytes were cultured before examined on productions of IFN- $\gamma$  by conventional enzyme-immunoassay to evaluate an inducing activity of production of IFN- $\gamma$ . The present polypeptide proved to act on the splenocytes, immunocompetent cells, to induce the production of IFN- $\gamma$ . The inducing activity of IFN- $\gamma$  production of the present polypeptide was equal to or higher than that of the wild-type polypeptide.

#### Example A-8(h)

#### Acute toxicity test

The present polypeptide in Example A-8(a) was examined on the acute toxicity by the method in Example A-1(j). As a result, the LD<sub>50</sub> of the present polypeptide proved to be about one mg or higher per one kg of the body weight, independently of the administration routs. This evidences that the present polypeptide can be incorporated into pharmaceuticals for mammalian including humans without anxiety.

### Example A-9

### 5 Production of polypeptide

An autonomously replicable recombinant DNA "pCSMIGIF/MUT12" containing the nucleotide sequence of SEQ ID NO:23 was obtained by a procedure similar as in Example A-8(a) but using the DNA fragment 9, obtained in Example

A-8(a), as a template, and an oligonucleotide with the nucleotide sequence of 5'-GGACACTTTCTT-GCTAGCCAAAAGG-3' and an oligonucleotide with the nucleotide sequence of 5'-CCTTTTGGCTAGCAAGAAAGT-GTCC-3', as a mutagenic sense and a mutagenic antisense primer, respectively, to replace the cysteine at 125th position in SEQ ID NO:5 with a serine. As shown in FIG. 13, in the recombinant DNA "pCSMIGIF/MUT12", a cDNA "mIGIF/MUT12" encoding the amino acid sequence of SEQ ID NO:14 was linked to downstream of the nucleotide sequence "IFNss", encoding the signal peptide of the subtype α2b of human interferon-α.

The recombinant DNA was introduced into COS-1 cells similarly as in Example A-1(b) to obtain a transformant. Culturing the transformant produced the polypeptide with the amino acid sequence of SEQ ID NO:14 in an amount of about 50 ng per one ml of the culture. The culture was purified before analyzed on the physicochemical properties similarly as in Example A-8. As a result, the polypeptide in this Example proved to be similar to that in Example A-8 in the properties, i.e., the molecular weight, the N-terminal amino acid sequence, and the less toxicity. As shown in FIG. 12, the results of the analysis on stability, obtained according to the method in Example A-8(f), the present polypeptide in this Example was more stable than the wild-type polypeptide. These results evidence that the amino acid replacement used in this Example can effectively improve the stability of the wild type polypeptide without reducing the biological activities.

#### Example B-1

#### Solution

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Any one of the present polypeptides purified in Examples A-1 to A-9 was dissolved in physiological saline containing one v/v % human serum albumin as a stabilizer to give a concentration of one mg/ml, and the solution was membrane-filtered in usual manner into a germ-free solution.

The solutions, with a satisfactory stability, can be used as an injection, an ophthalmic solution, and a collunarium for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections and immunopathies, of mammalian including human.

#### Example B-2

### 30 Dry injection

One hundred mg of any one of the present polypeptides purified in Examples A-1 to A-9 was dissolved in 100 ml of physiological saline containing one w/v % gelatin as a stabilizer, and the solution was sterilized membrane-filtered in usual manner into a germ-free solution. One ml aliquotes of each of the sterilized solutions were distributed to vials, and lyophilized before sealing the vials with caps.

The products, with a satisfactory stability, can be used as a dry injection for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections, and immunopathies of mammalian including human.

#### Example B-3

Ointment

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"HI-BIS-WAKO 104", a carboxyvinylpolymer commercialized by Wako Pure Chemicals, Tokyo, Japan, and "TRE-HALOSE", a powdered crystalline trehalose commercialized by Hayashibara Co., Ltd., Okayama, Japan, were dissolved in sterilized distilled water to give concentrations of 1.4 w/w % and 2.0 w/w %, respectively. Any one of the present polypeptides purified in Examples A-1 to A-9 was mixed with the solution into homogeneity. Each of the homogenate was adjusted to pH 7.2 to obtain a paste containing about one mg/g of any one of the polypeptides.

The pastes, with a satisfactory spreadablity and stability, can be used as an ointment for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections and immunopathies, of mammalian including human.

#### Example B-4

#### Tablet

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Any one of the purified polypeptides in Examples A-1 to A-9 and "LUMIN", [bis-4-(1-ethylquinoline)][ $\gamma$ -4'-(1-ethylquinoline)] pentamethionine cyanine, as a cell activator, were mixed with "FINETOSE®, an anhydrous crystalline  $\alpha$ -maltose commercialized by Hayashibara Co., Ltd., Okayama, Japan, into homogeneity. Each of the homogenate was

processed with a conventional tablet machine into tablets, each of which weighed 200 mg and contained about one mg of any of the polypeptides and the LUMIN.

The tablets with a satisfactory swallowability, stability and cell-activating activity can be used as a tablet for treating and/or preventing susceptive diseases such as malignant tumors, viral diseases, infections and immunopathies, of mammalian including human.

#### Example B-5

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### Adoptive immunotherapeutic agent

Mononuclear cells were isolated from a peripheral blood of a patient with malignant lymphoma. The cells were suspended in RPMI-1640 medium supplemented with 10 v/v % human AB serum, preheated at 37°C, to give a density of 1×10<sup>6</sup> cells/ml. To the cell suspension, any one of the present polypeptides in Examples A-1 to A-7 and a recombinant human interleukin 2 were added as adoptive immunotherapeutic agent to give concentrations of 10 ng/ml and 100 units/ml, respectively, before the cells were cultured at 37°C for one week in a 5 v/v % CO<sub>2</sub> incubator. Thereafter, the culture was centrifuged to collect LAK cells.

The LAK cells can exhibit so strong cytotoxicity to the lymphoma when returned to the patient, and an adoptive immunotherapy using the present agent can exert significantly higher effect than that using the interleukin 2 alone. Cytotoxic T cells obtained similarly as above excepting the mononuclear cells, replaced with tumor-invasive lymphocytes, also can effect as equivalent to that of the LAK cells, when returned to the patient. Thus the adoptive immunotherapeutic agent in this Example can be effectively applied to solid malignant tumors such as renal cancer, malignant melanoma, colonic cancer, rectal cancer, and lung cancer, besides malignant lymphomas.

IFN-y is well known to be involved in protection against infections of virus and bacteria, etc., inhibition of malignant tumors proliferation, regulation of immune system causing protection, and inhibition of immunoglobulin E antibodies production. And IFN-y is now in use for agents against human susceptive diseases, stating that the directions for the targeting diseases, uses, dosages, and safeness have been already established.

As described in a publication as Frances R. Balkwill, *Saitokain-To-Ganchiryo (Cytokines in Cancer Therapy)*, Yoshihiko WATANABE tr., (Tokyo, Japan: Tokoyo Kagaku Dojin Co., Ltd., 1991), therapies using killer cells such as NK cells and LAK cells that include antitumor immunotherapies are applied to human diseases, resulting in satisfactory effects as a whole. Recently, an intensive interest is taken in the involvement of the killer cells, which have cytotoxicities enhanced by cytokines, or which are formed induced by cytokines, in therapeutic effects. For example, T. Fujioka et al., *British Journal of Urology*, Vol.73, No.1, pp.23-31 (1994) describes that in an antitumor immunotherapy using both LAK cells and interleukin 2, the interleukin 2 induced formation of the LAK cells, resulting in remarkable effects against human cancer metastases without exhibiting serious toxicities and side effects.

Thus, it has been revealed that IFN- $\gamma$  or killer cells are involved in treatment and/or prevention of a variety of human diseases, and can contribute to cure or remission to the diseases. As shown in Examples A-1 to A-9, the present polypeptides induce the production of IFN- $\gamma$  by immunocompetent cells, enhance the cytotoxicity of NK cells, and induce the formation of LAK cells, indicating that the present agents for susceptive diseases can be administered to patients successively for a relatively-long period of time, and effect to treat and/or prevent diseases, in which IFN- $\gamma$  and/or killer cells are involved, without causing serious side effects.

### [Effect of the invention]

As described above, the present invention is made based on the establishment of stable polypeptides capable of inducing production of IFN- $\gamma$  by immunocompetent cells. The polypeptides according to the present invention are the substances clarified on their amino acid sequence, and feature to retain the biological activities for a relatively-long period in actual use, because of the higher stability than that of the wild-type polypeptide. Thus the present polypeptides provide a variety of uses such as an IFN- $\gamma$  inducer for producing IFN- $\gamma$  in cell cultures and an agent for treating and/or preventing diseases sensitive to IFN- $\gamma$  in general, including viral diseases, infections, malignant tumors, and immunopathies. The agents with the present polypeptides additionally possessing properties of enhancing cytotoxicities and/or inducing formation of killer cells, as effective ingredients, can satisfactorily treat serious diseases such as malignant tumors.

Furthermore, the present polypeptides generally can induce a desired level of IFN- $\gamma$  with only a slight amount since they have so strong activity of inducing production of IFN- $\gamma$ . Because of little toxicity, the polypeptides wouldn't cause serious side effects even when administered with relatively-high doses. These give the present polypeptides an advantage of that they can induce a desired level of IFN- $\gamma$  rapidly without strictly controll on the dosages in actual use. The polypeptides with these usefulness can be easily produced in a desired amount by the present process using recombinant DNA techniques.

The present invention is a significant invention which has a remarkable effect and gives a great contribution to this field

While there has been described what is at present considered to be the preferred embodiments of the present invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

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### SEQUENCE LISTING

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(1) GENA	RAL IN	FORMATION:
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- (iii) NUMBER OF SEQUENCES:30
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  - (F) POSTAL CODE (ZIP):700
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE:Floppy disk
  - (B) COMPUTER: IBM compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: JP 333,037/96
  - (B) FILING DATE: November 29, 1996
  - (A) APPLICATION NUMBER: JP 20,906/97
  - (B) FILING DATE: January 21, 1997
  - (A) APPLICATION REFERENCE NO: JP 10,053,503
  - (B) FILING DATE: November 14, 1997
- (2) INFORMATION FOR SEQ ID NO:1:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 6 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE: peptide
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
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  - (3) INFORMATION FOR SEQ ID NO:2:
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      - (A) LENGTH: 6 amino acids
      - (B) TYPE: amino acid
      - (D) TOPOLOGY: linear
    - (ii) MOLECULE TYPE: peptide
    - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Phe Glu Asp Met Thr Asp
1 5

- (4) INFORMATION FOR SEO ID NO:3:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH:5 amino acids

(B) TYPE: amino acid

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(D) TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
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                   (B) TYPE: amino acid
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                   (D) TOPOLOGY: linear
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                                  55
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                                              90
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      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
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(8) INFORMATION FOR SEQ ID NO:7:
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          (B) TYPE: amino acid
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      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
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                   (B) TYPE: amino acid
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              (ii) MOLECULE TYPE: peptide
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        Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
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        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
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        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
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        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
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        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
30
                                 135
        Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
        145
                             150
35
        (10) INFORMATION FOR SEQ ID NO:9:
              (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
        Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
        Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
45
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
50
                             70
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                    100
                                         105
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
```

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
130 135 140
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
145 150 155

(11) INFORMATION FOR SEQ ID NO:10:

10

35

40

50

55

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 157 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 15 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 20 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 25 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu . 135 30 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

- (12) INFORMATION FOR SEQ ID NO:11:
  - (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH:157 amino acids
    - (B) TYPE: amino acid
    - (D) TOPOLOGY: linear
  - (ii) MOLECULE TYPE:peptide
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
        145
                             150
5
         (13) INFORMATION FOR SEQ ID NO:12:
               (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
10
               (ii) MOLECULE TYPE: peptide
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
        Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
15
        Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
20
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
25
                                         105
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                      120
        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                 135
        Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
30
                             150
                                                  155
         (14) INFORMATION FOR SEQ ID NO:13:
               (i) SEQUENCE CHARACTERISTICS:
35
                   (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                   (D) TOPOLOGY: linear
               (ii) MOLECULE TYPE:peptide
               (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
40
        Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn
        Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
        Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
45
        Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
        Val Lys Asp Ser Lys Met Ser Thr Lou Ser Cys Lys Asn Lys Ile Ile
                             70
        Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
50
        Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
                     100
                                          105
        Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
                                     120
                                                          125
55
        Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
             130
```

Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser

150 . 5 (15) INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 157 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear 10 (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14: Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 20 70 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 100 105 25 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Ser Gln Lys Glu 120 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 135 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 145 150 30 (16) INFORMATION FOR SEQ ID NO:15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 471 base pairs 35 (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE:cDNA (ix) FEATURE: (A) NAME/KEY: mat peptide (B) LOCATION: 1..471 (C) IDENTIFICATION METHODS:S (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15: TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT 45 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arq Asn Leu Asn 10 GAC CAA GTT CTC TTC ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT 96 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 25 ATG ACT GAT TCT GAC TGT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 TCT GTG AAG TCT GAG AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile

	65					70					75					80	
_	ATT Ile	TCC Ser	TTT Phe	AAG Lys	GAA Glu 85	ATG Met	AAT Asn	CCT Pro	CCT Pro	GAT Asp 90	AAC Asn	ATC Ile	AAG Lys	GAT Asp	ACA Thr 95	AAA Lys	288
<i>5</i>	AGT Ser	GAC Asp	ATC Ile	Ile	TTC	TTT Phe	CAG Gln	AGA Arg	Ser	GTC	CCA Pro	GGA Gly	CAT His	Asp	AAT	AAG Lys	336
	ATG Met	CAA Gln	Phe	100 GAA Glu	TCT Ser	TCA Ser	TCA Ser	Tyr	GAA Glu	GGA Gly	TAC Tyr	TTT Phe	Leu	GCT Ala	TGT Cys	GAA Glu	384
10	AAA Lys	GAG Glu	115 AGA Arg	GAC Asp	CTT Leu	TTT Phe	Lys	120 CTC Leu	ATT Ile	TTG Leu	AAA Lys	AAA Lys	125 GAG Glu	GAT Asp	GAA Glu	TTG Leu	432
15		130 GAT Asp															471
15	145					150					155		-				
	(17)		FORM i)SE(														
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		()		C) IDE	CATIO ENTII NCE I	FICAT	NOIT	METH			) • 16 •	,					
30		TTT Phe															48
		CAA Gln															96
35		ACT Thr	Asp	TCT				Asp	AAT				Thr	ATA			144
		AGT															192
40		Ser 50					55					60					
40	TCT Ser 65	GTG Val	AAG Lys	TCT Ser	GAG Glu	AAA Lys 70	ATT	TCA Ser	ACT Thr	CTC Leu	TCC Ser 75	TGT Cys	GAG Glu	AAC Asn	AAA Lys	ATT Ile 80	240
		TCC Ser			Glu					Asp					Thr	AAA	288
45		GAC Asp		Ile													336
	ATG	CAA	TTT	100 GAA	TCT	TCA	TCA	TAC	105 GAA	GGA	TAC	TTT	CTA	110 GCT	TGT	GAA	384
50 .	Met	Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu	
		GAG Glu 130															432
55		GAT Asp					TTC					GAA					471

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(18) INFORMATION FOR SEQ ID NO:17:
           (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 471 base pairs
               (B) TYPE: nucleic acid
5
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear
           (ii) MOLECULE TYPE: cDNA
           (iX) FEATURE:
               (A) NAME/KEY: mat peptide
10
               (B) LOCATION:1..471
               (C) IDENTIFICATION METHODS: S
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
     TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
                                                                            48
     Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
15
                                          10
     GAC CAA GTT CTC TTC ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT
                                                                            96
     Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                 20
                                      25
     ATG ACT GAT TCT GAC TGT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT
                                                                           144
20
    Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
                                  40
     ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC
                                                                           192
     Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                              55
     TCT GTG AAG TCT GAG AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT
                                                                           240
25
     Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
                         70
                                              75
     ATT TCC TTT AAG GAA ATG AAT CCT CCT GAT AAC ATC AAG GAT ACA AAA
                                                                           288
     Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                     85
                                          90
30
    AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAT AAG
                                                                          336
    Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                                      105
     ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TCT GAA
                                                                          384
    Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
             115
                                  120
                                                      125
35
    AAA GAG AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG
                                                                           432
    Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                             135
                                                  140
    GGG GAT AGA TCT ATA ATG TTC ACT GTT CAA AAC GAA GAC
                                                                          471
    Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
40
     145
     (19) INFORMATION FOR SEQ ID NO:18:
           (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 471 base pairs
45
               (B) TYPE: nucleic acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: linear
           (ii) MOLECULE TYPE: cDNA
           (iX) FEATURE:
               (A) NAME/KEY: mat peptide
50
               (B) LOCATION: 1..471
               (C) IDENTIFICATION METHODS:S
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
    TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
                                                                           48
    Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
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	1				5					10			•		15		
	·GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
	Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
_				20					25		_			30		•	
5	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arq	Thr	Ile	Phe	Ile	
			35		-		•	40				-	45				
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	Ile	Ser	Met	Tyr	Lvs	Asp	Ser	Gln	Pro	Ara	Glv	Met	Ala	Val	Thr	Tle	
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				Ser													240
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				Lys													288
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	аст	GAC	እ TC	ATA		ттт	CAG	AG A	እርጥ		CCA	CCA	CAT	CAT	95	3 3 C	226
	Sor	7020	Tlo	Ile	Pho	Dho	Cln	y ~ ~	SO.	W-1	DYA	Clu	UAI	GAI	AAI	AAG	336
	261	Asp	116	100	FILE	FILE	GIII	Arg		vai	PIO	Gry	uis	_	ASII	rås	
	A TIC	CAA	T T T	GAA	TOT	TON	TON	TAC	105	CCA	መክሮ	mmm	Cm N	110	mam		
00																	384
20	Met	GIII		Glu	ser	Ser	Ser		GIU	GIA	Tyr	Pne		АТА	ser	GLu	
	***	CAC	115	CNC	COM	mmm	222	120	A COO	ana.			125	a	~		
				GAC													432
	ьуs		Arg	Asp	Leu	Pne		ьeu	rre	Leu	rys		Glu	Asp	Glu	Leu	
	~~~	130	. ~ .				135					140					
25				TCT													471
		Asp	Arg	Ser			Pne	Thr	vai	GIn		Glu	Asp				
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30	(20)		i)SE(	QUEN	CE CI	IARA(	TER	STIC	CS:								
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30	(20)		i) se( [] (! ()	QUENC A) LEI B) TYI C) STI	CE CI NGTH: PE:ni RANDI	HARAC :471 uclei EDNES	Dase base c ac	STIC pai cid ouble	CS: .rs								
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	(20)	(: (:	L) SE( (1 (1 (1 (1) M( Li) M( LX) FI	QUENCA) LEI B) TYI C) STI O) TOI OLECT EATUI A) NAI	CE CH NGTH: PE:nu RANDH POLOC JLE T RE: ME/KH	HARAC :471 :471 :Clei :EDNES :GY:li :TYPE:	DTERI base ic ac SS:do inear cDN/	STICE paid cid cuble couble co	CS: rs								
	(20)	(: (:	L) SE( (1 (1 (1 (1) M( Li) M( LX) FI	QUENCA) LEI B) TYI C) STI C) TOI OLECU	CE CH NGTH: PE:nu RANDH POLOC JLE T RE: ME/KH	HARAC :471 :471 :Clei :EDNES :GY:li :TYPE:	DTERI base ic ac SS:do inear cDN/	STICE paid cid cuble couble co	CS: rs								
	(20)	(: (:	() SE( () () () () () () () () ()	QUENG A) LEI B) TYI C) STI OLECU EATUI A) NAI B) LOO C) IDI	CE CH NGTH: PE: nu RANDH POLOC JLE C RE: ME/KH CATIC	HARAC :471 :2Clei :EDNES GY:li :TYPE: EY:ma DN:l. FICAT	base ic ac is de inear cDNA at pe	ISTIC e pai cid ouble	CS: rs de								
	(20)	(: (:	() SE( () () () () () () () () ()	QUENCA) LEIGHT (CONTROL CONTROL CONTRO	CE CH NGTH: PE: nu RANDH POLOC JLE C RE: ME/KH CATIC	HARAC :471 :2Clei :EDNES GY:li :TYPE: EY:ma DN:l. FICAT	base ic ac is de inear cDNA at pe	ISTIC e pai cid ouble	CS: rs de		):19:	·					
		(: (: (:	() SE( () () () () () () () () () () () () ()	QUENCA) LEI B) TYI C) STI C) STI OLECU EATUI A) NAI A) LOC C) IDI EQUEI	CE CH NGTH: PE: no RANDI POLOC JLE C RE: ME/KI CATIC ENTII NCE I	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCE	base base construction to the construction to	STICE PAIR CON:S	CS: Trs de de dobs:	D NC							
35	TAC	(; (; (; TTT	() SE( () () () () () () () () () () () () ()	QUENCA) LEI B) TYI C) STI C) STI C) TOI DLECT EATUI A) NAI B) LOC C) IDI EQUEI	CE CE NGTH: PE: NO RE: POLOCI JLE CO RE: ME/KE CATICE VCE I	HARAC :471 IClei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCE	base base construction to the construction to	STICE PAIR CON:S	CS: Trs de de dods: SEQ d	D NO	GTC	ATA					48
35	TAC	(; (; (; TTT	() SE( () () () () () () () () () () () () ()	QUENCA) LEI B) TYI C) STI C) STI OLECU EATUI A) NAI A) LOC C) IDI EQUEI	CE CE NGTH: PE: NO RE: POLOCI JLE CO RE: ME/KE CATICE VCE I	HARAC :471 IClei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCE	base base construction to the construction to	STICE PAIR CON:S	CS: Trs de de dods: SEQ d	D NO	GTC	ATA					<b>48</b>
35	TAC Tyr 1	(: (: TTT Phe	i)SEC (I (I (I (I (I)MC (IX)FI (I (C (I)SI GGC Gly	QUENCA) LEIB B) TYI C) STI C) STI C) TOI CLECT CLEATUR A) NAM B) LOC C) IDI EQUER AAG LYS	CE CH NGTH: PE:nu RANDI POLOCULE TO RE: ME/KH CATIC ENTII NCE I CTT Leu 5	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu	base converse per	STICE PAIR CID DUBLE CON:S AAA Lys	CS: Trs de de MODS: EEQ 1	TCA Ser 10	GTC Val	ATA Ile	Arg	Asn	Leu 15	Asn	<b>48</b>
35	TAC Tyr 1 GAC	(: (: TTT Phe	i) SEQ (I (I (I (I (I (I (I (I (I (I (I (I (I	QUENCA) LEIB B) TYII C) STII C) STII C) TOI C) LOI EATUI A) NAM B) LOO C) IDII EQUEI AAG LYS	CE CH NGTH: PE:nu RANDI POLOCI JLE T RE: ME/KH CATIC ENTIH NCE I CTT Leu 5	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:1. FICAT DESCR GAA Glu	base converse convence converse converse converse converse converse converse convers	STICE paid cid buble for the constant of the c	irs de dobs: TTA Leu GGA	TCA Ser 10 AAT	GTC Val CGG	ATA Ile CCT	Arg CTA	Asn TTT	Leu 15 GAA	Asn GAT	48
35	TAC Tyr 1 GAC	(: (: TTT Phe	i) SEQ (I (I (I (I (I (I (I (I (I (I (I (I (I	QUENCA) LEIB B) TYI C) STI C) STI C) TOI CLECT CLEATUR A) NAM B) LOC C) IDI EQUER AAG LYS	CE CH NGTH: PE:nu RANDI POLOCI JLE T RE: ME/KH CATIC ENTIH NCE I CTT Leu 5	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:1. FICAT DESCR GAA Glu	base converse convence converse converse converse converse converse converse convers	STICE paid cid buble for the constant of the c	irs de dobs: TTA Leu GGA	TCA Ser 10 AAT	GTC Val CGG	ATA Ile CCT	Arg CTA	Asn TTT	Leu 15 GAA	Asn GAT	
35	TAC Tyr 1 GAC Asp	(: (: TTT Phe CAA Gln	i) SEQ (I (I (I (I (I (I (I (I (I (I (I (I (I	QUENCA) LEIB B) TYII C) STII C) STII C) TOI C) LOI EATURA) NAM B) LOC C) IDI EQUEN AAG Lys CTC Leu 20	CE CH NGTH: PE:nu RANDI POLOCI JLE T RE: ME/KH CATIC ENTIH NCE I CTT Leu 5 TTC Phe	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:1. FICAT DESCR GAA Glu ATT Ile	base converse convence converse converse converse converse converse converse convers	ESTICE paid cid buble for the constant of the	S: Irs de MODS: EEQ I TTA Leu GGA Gly 25	TCA Ser 10 AAT Asn	GTC Val CGG Arg	ATA Ile CCT Pro	Arg CTA Leu	Asn TTT Phe 30	Leu 15 GAA Glu	Asn GAT Asp	
35	TAC Tyr 1 GAC Asp	(: (: TTT Phe CAA Gln	i) SEQ (I (I (I (I (I (I (I (I (I (I (I (I (I	QUENCA) LED B) TYD C) STI C) STI C) TOD C) TOD EATUM A) NAM B) LOC C) IDM EQUEN AAG Lys CTC Leu	CE CH NGTH: PE:nu RANDI POLOCI JLE T RE: ME/KH CATIC ENTIH NCE I CTT Leu 5 TTC Phe	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:1. FICAT DESCR GAA Glu ATT Ile	base converse convence converse converse converse converse converse converse convers	ESTICE paid cid buble for the constant of the	S: Irs de MODS: EEQ I TTA Leu GGA Gly 25	TCA Ser 10 AAT Asn	GTC Val CGG Arg	ATA Ile CCT Pro	Arg CTA Leu	Asn TTT Phe 30	Leu 15 GAA Glu	Asn GAT Asp	
35	TAC Tyr 1 GAC Asp	(: (: TTT Phe CAA Gln	i) SEQ (I) (I) (I) (I) (I) (I) (I) (I) (I) (I)	QUENCA) LEIB B) TYII C) STII C) STII C) TOI C) LOI EATURA) NAM B) LOC C) IDI EQUEN AAG Lys CTC Leu 20	CE CH NGTH: PE:nu RANDI POLOCI JLE T RE: ME/KH CATIC ENTIH VCE I CTT Leu 5 TTC Phe GAC	HARAC :471 uclei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCH GAA Glu ATT Ile TCT	base ic ac SS:do inean cDNA it pe .471 TION TCT Ser GAC Asp	STICE paid cid buble cid buble cid buble cid buble cid cid buble cid	ICS: ITS IC ICDS: ICA ICA ICA ICA ICA ICA ICA ICA ICA ICA	TCA Ser 10 AAT Asn	GTC Val CGG Arg	ATA Ile CCT Pro	Arg CTA Leu ACC	Asn TTT Phe 30 ATA	Leu 15 GAA Glu TTT	Asn GAT Asp ATT	96
35	TAC Tyr 1 GAC Asp ATG Met	(; (; TTT Phe CAA Gln ACT Thr	i) SEQ (I) (I) (I) (I) (I) (I) (I) (I) (I) (I)	QUENCA) LED B) TYD C) STI C) STI C) TOD C) TOD EATUM A) NAM B) LOC C) IDM EQUEN AAG Lys CTC Leu 20 TCT Ser	CE CE NGTH: PE:nu RANDI POLOO JLE T RE/KE CATIO CATIO CTT Leu 5 TTC Phe GAC Asp	HARAC :471 iclei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser	base ic ac SS:do inean cDNA it pe .471 TION TCT Ser GAC Asp	ESTICE paid cid buble for the constant of the	ICS: Irs Ide IODS: IEQ ITTA Leu IGGA Gly 25 AAT Asn	TCA Ser 10 AAT ASD GCA Ala	GTC Val CGG Arg CCC Pro	ATA Ile CCT Pro CGG Arg	Arg CTA Leu ACC Thr 45	TTT Phe 30 ATA Ile	Leu 15 GAA Glu TTT Phe	Asn GAT Asp ATT Ile	96
35	TAC Tyr 1 GAC Asp ATG Met	(; (; TTT Phe CAA Gln ACT Thr	i) SEQ (I) (I) (I) (I) (I) (I) (I) (I) (I) (I)	QUENCA) LEIM B) TYI C) STI C) STI C) TOI CLECT CATUMA A) NAM B) LOC C) IDM EQUEM AAG Lys CTC Leu 20 TCT	CE CE NGTH: PE:nu RANDI POLOG JLE 7 RE: ME/KE CATIC CATIC CTT Leu 5 TTC Phe GAC Asp	HARAC :471 iclei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser	base ic ac SS:do inean cDNA it pe .471 TION TCT Ser GAC Asp	ESTICE paid cid buble for the constant of the	ICS: Irs Ide IODS: IEQ ITTA Leu IGGA Gly 25 AAT Asn	TCA Ser 10 AAT ASD GCA Ala	GTC Val CGG Arg CCC Pro	ATA Ile CCT Pro CGG Arg	Arg CTA Leu ACC Thr 45	TTT Phe 30 ATA Ile	Leu 15 GAA Glu TTT Phe	Asn GAT Asp ATT Ile	96
35	TAC Tyr 1 GAC Asp ATG Met	(: (: TTT Phe CAA Gln ACT Thr	i) SEQ (I (I (I (I) MO (IX) FI (I) (I) (I) (I) (I) (I) (I) (I) (I) (I	QUENCA) LED B) TYD C) STI C) STI C) TOD C) TOD EATUM A) NAM B) LOC C) IDM EQUEN AAG Lys CTC Leu 20 TCT Ser	CE CE NGTH: PE:nu RANDI POLOO JLE 7 RE: ME/KE CATIC CATIC CTT Leu 5 TTC Phe GAC ASP	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT	base ic ac SS:dc inean cDNA at pe .471 TION TCT Ser GAC ASP AGA AGA	ESTICE paid cid buble for the constant of the	ICS: Irs Ide IODS: IEQ ITTA Leu GGA Gly 25 AAT Asn	TCA Ser 10 AAT Asn GCA Ala	GTC Val CGG Arg CCC Pro	ATA Ile CCT Pro CGG Arg	Arg CTA Leu ACC Thr 45 GCT	TTT Phe 30 ATA Ile	Leu 15 GAA Glu TTT Phe	Asn GAT Asp ATT Ile ATC	· 96
35 40 45	TAC Tyr 1 GAC Asp ATG Met	(: (: TTT Phe CAA Gln ACT Thr AGT Ser	i) SEQ (I (I (I (I) M(I (IX) FI (I) (I) (I) (I) (I) (I) (I) (I) (I) (I	QUENCA) LED B) TYD C) STI C) STI C) STI C) TOD EATUM A) NAM B) LOC C) IDM EQUEN AAG Lys CTC Leu 20 TCT Ser TAT Tyr	CE CENGTH: PE:nu RANDI POLOG JLE T RE-KE CATIC ENTIE VCE I CTT Leu 5 TTC Phe GAC ASP AAA Lys	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT Asp	base ic ac SS:do inean cDNA at pe .471 TION TCT Ser GAC Asp AGA Arg	ESTICE paid cid buble for the constant of the	Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic I	TCA Ser 10 AAT ASN GCA Ala AGA	GTC Val CGG Arg CCC Pro	ATA Ile CCT Pro CGG Arg ATG Met 60	Arg CTA Leu ACC Thr 45 GCT Ala	TTT Phe 30 ATA Ile GTA Val	Leu 15 GAA Glu TTT Phe ACT Thr	Asn GAT Asp ATT Ile ATC	· 96
35 40 45	TAC Tyr 1 GAC Asp ATG Met ATA Ile	(: (: TTT Phe CAA Gln ACT Thr AGT Ser 50 GTG	i) SEQ (I (I (I (I) MO (IX) FI (I (I) C (I) SI GGC Gly GTT Val GAT Asp 35 ATG Met	QUENCA) LEIN B) TYI C) STI C) STI C) TOI C) LECT CA) NAM B) LOC C) IDI C) LOC LYS CTC Leu 20 TCT Ser TAT TYT	CE CENGTH: PE:nu PE:nu PE:nu POLOO JLE T RE/KE CATIO CATIO CTT Leu S TTC Phe GAC ASP AAA Lys GAG	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT ASP AAA	base ic ac is side inean ic DNA at pe .471 TION TCT Ser GAC ASP AGA Arg	ESTICE paid cid buble for the paid cid buble	Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic I	TCA Ser 10 AAT ASN GCA Ala AGA Arg	GTC Val CGG Arg CCC Pro GGT Gly	ATA Ile CCT Pro CGG Arg ATG Met 60 TCT	Arg CTA Leu ACC Thr 45 GCT Ala	TTT Phe 30 ATA Ile GTA Val	Leu 15 GAA Glu TTT Phe ACT Thr	Asn GAT Asp ATT Ile ATC Ile ATT	· 96
35 40 45	TAC Tyr 1 GAC Asp ATG Met ATA Ile	(: (: TTT Phe CAA Gln ACT Thr AGT Ser 50 GTG	i) SEQ (I (I (I (I) MO (IX) FI (I) (I) (I) (I) (I) (I) (I) (I) (I) (I	QUENCA) LEIN B) TYI C) STI C) STI C) TOI C) LECT CA) NAM B) LOC C) IDI C) LOC LYS CTC Leu 20 TCT Ser TAT TYT	CE CENGTH: PE:nu PE:nu PE:nu POLOO JLE T RE/KE CATIO CATIO CTT Leu S TTC Phe GAC ASP AAA Lys GAG	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT ASP AAA	base ic ac is side inean ic DNA at pe .471 TION TCT Ser GAC ASP AGA Arg	ESTICE paid cid buble for the paid cid buble	Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic I	TCA Ser 10 AAT ASN GCA Ala AGA Arg	GTC Val CGG Arg CCC Pro GGT Gly	ATA Ile CCT Pro CGG Arg ATG Met 60 TCT	Arg CTA Leu ACC Thr 45 GCT Ala	TTT Phe 30 ATA Ile GTA Val	Leu 15 GAA Glu TTT Phe ACT Thr	Asn GAT Asp ATT Ile ATC Ile ATT	96 144 192
35 40 45	TAC Tyr 1 GAC Asp ATG Met ATA Ile	(: (: TTT Phe CAA Gln ACT Thr AGT Ser 50 GTG	i) SEQ (I (I (I (I) MO (IX) FI (I) (I) (I) (I) (I) (I) (I) (I) (I) (I	QUENCA) LED B) TYD C) STI C) STI C) STI C) TOD EATUM A) NAM B) LOC C) IDM EQUEN AAG Lys CTC Leu 20 TCT Ser TAT Tyr	CE CENGTH: PE:nu PE:nu PE:nu POLOO JLE T RE/KE CATIO CATIO CTT Leu S TTC Phe GAC ASP AAA Lys GAG	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT ASP AAA	base ic ac is side inean ic DNA at pe .471 TION TCT Ser GAC ASP AGA Arg	ESTICE paid cid buble for the paid cid buble	Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic Ic I	TCA Ser 10 AAT ASN GCA Ala AGA Arg	GTC Val CGG Arg CCC Pro GGT Gly	ATA Ile CCT Pro CGG Arg ATG Met 60 TCT	Arg CTA Leu ACC Thr 45 GCT Ala	TTT Phe 30 ATA Ile GTA Val	Leu 15 GAA Glu TTT Phe ACT Thr	Asn GAT Asp ATT Ile ATC Ile ATT	96 144 192
35 40 45	TAC Tyr 1 GAC Asp ATG Met ATA Ile TCT Ser 65	(: (: TTT Phe CAA Gln ACT Thr AGT Ser 50 GTG Val	i) SEQ (I) (I) (I) (I) (I) (I) (I) (I) (I) (I)	QUENCA) LEIN B) TYI C) STI C) STI C) TOI C) LECT CA) NAM B) LOC C) IDI C) LOC LYS CTC Leu 20 TCT Ser TAT TYT	CE CENGTH: PE:nu P	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT Asp AAA Lys 70	base ic ac is side inean ic DNA at pe .471 TION TCT Ser GAC ASP AGA Arg AGC Ser 55 ATT Ile	ESTICE paid ouble of the paid ouble outle outle outle ouble outle	Ics: Irs Ics Ics Ics Ics Ics Ics Ics Ics Ics Ic	TCA Ser 10 AAT ASN GCA Ala AGA Arg CTC Leu	GTC Val CGG Arg CCC Pro GGT Gly TCC Ser 75	ATA Ile CCT Pro CGG Arg ATG Met 60 TCT Ser	Arg CTA Leu ACC Thr 45 GCT Ala GAG Glu	Asn TTT Phe 30 ATA Ile GTA Val AAC Asn	Leu 15 GAA Glu TTT Phe ACT Thr	Asn GAT Asp ATT Ile ATC Ile ATT Ile 80	144 192 -
35 40 45	TAC Tyr 1 GAC Asp ATG Met ATA Ile TCT Ser 65 ATT	(: (: TTT Phe CAA Gln ACT Thr AGT Ser 50 GTG Val	i) SEQ (I) (I) (I) (I) (I) (I) (I) (I) (I) (I)	QUENCA) LEIN B) TYI C) STI C) STI C) TOI CLECT CA) NAM B) LOC C) IDE C LYS CTC Leu 20 TCT Ser TAT TYT Ser AAG	CE CE CENGTH: PE: nu PE	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT ASP AAA Lys 70 ATG	DASSIC AGENTAL SET AGA AGA AGA AGA ATT ILE	ESTICE paid ouble of the paid ouble of the paid ouble of the paid ouble of the paid ouble outle	de GGA Gly 25 AAT Asn CCT Thr CCT	TCA Ser 10 AAT ASN GCA Ala AGA Arg CTC Leu	GTC Val CGG Arg CCC Pro GGT Gly TCC Ser 75 AAC	ATA Ile CCT Pro CGG Arg ATG Met 60 TCT Ser	Arg CTA Leu ACC Thr 45 GCT Ala GAG Glu AAG	Asn TTT Phe 30 ATA 11e GTA Val AAC Asn GAT	Leu 15 GAA Glu TTT Phe ACT Thr AAA Lys	Asn GAT Asp ATT Ile ATC Ile ATT Ile 80 AAA	96 144 192
35 40 45	TAC Tyr 1 GAC Asp ATG Met ATA Ile TCT Ser 65 ATT	(: (: TTT Phe CAA Gln ACT Thr AGT Ser 50 GTG Val	i) SEQ (I) (I) (I) (I) (I) (I) (I) (I) (I) (I)	QUENCA) LEIN B) TYI C) STI C) STI C) TOI CLECT CANNAM B) LOC C) IDE COUEN ANG Lys CTC Leu 20 TCT Ser TAT TYT Ser	CE CE CENGTH: PE: nu PE	HARAC 471 101ei EDNES GY:li TYPE: EY:ma DN:l. FICAT DESCR GAA Glu ATT Ile TCT Ser GAT ASP AAA Lys 70 ATG	DASSIC AGENTAL SET AGA AGA AGA AGA ATT ILE	ESTICE paid ouble of the paid ouble of the paid ouble of the paid ouble of the paid ouble outle	de GGA Gly 25 AAT Asn CCT Thr CCT	TCA Ser 10 AAT ASN GCA Ala AGA Arg CTC Leu	GTC Val CGG Arg CCC Pro GGT Gly TCC Ser 75 AAC	ATA Ile CCT Pro CGG Arg ATG Met 60 TCT Ser	Arg CTA Leu ACC Thr 45 GCT Ala GAG Glu AAG	Asn TTT Phe 30 ATA 11e GTA Val AAC Asn GAT	Leu 15 GAA Glu TTT Phe ACT Thr AAA Lys	Asn GAT Asp ATT Ile ATC Ile ATT Ile 80 AAA	144 192 -

	AGT Ser	GAC Asp	ATC Ile	ATA Ile	TTC Phe	TTT Phe	CAG Gln	AGA Arg	AGT Ser	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp	AAT Asn	AAG Lvs	336
				100					105					110		•	
5	ATG Met	CAA Gln	TTT Phe	GAA Glu	TCT Ser	TCA Ser	TCA Ser	TAC Tyr	GAA Glu	GGA Gly	TAC Tyr	TTT Phe	CTA Leu	GCT Ala	TCT Ser	GAA Glu	384
			115					120					125				
	AAA Lys	GAG Glu 130	AGA Arg	GAC Asp	CTT Leu	TTT Phe	AAA Lys 135	CTC Leu	ATT Ile	TTG Leu	AAA Lys	Lys	GAG Glu	GAT Asp	GAA Glu	TTG Leu	432
10	ccc		AGA	тст	ATA	ΔТС		ΔСΤ	CTT	ראא	አአሮ	140	CAC				471
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					CTT Leu												48
	1	FILE	Gry	nys	5	Gru	Ser	цув	пец	10	vai	TIE	Arg	ASII	15	ASI	
	_	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA		CGG	ССТ	CTA	TTT		GAT	96
30					Phe												, ,
55	_			20			_		25		_			30		•	
					GAC												144
	Met	Thr		Ser	Asp	Ser	Arg		Asn	Ala	Pro	Arg		Ile	Phe	Ile	
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35					Lys												192
		50		-1-	<i> y u</i>		55	<b></b>		••• 5	<b>-</b> 1	60		V W 1	****	110	
	TCT		AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
	Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
	65																
40					GAA												288
	Tie	ser	Pne	rys	Glu 85	мес	Asn	Pro	Pro	ASP 90	ASN	TTE	rys	Asp		Lys	
	AGT	GAC	ATC	ΔΤΔ	TTC	ጥጥጥ	CAG	AGA	AGT		CCA	GG A	СДТ	СУТ	95 887	AAG	336
					Phe												330
•		•		100				,	105					110		-1-	
45					TCT												384
	Met	Gln		Glu	Ser	Ser	Ser		Glu	Gly	Tyr	Phe		Ala	Cys	Glu	
			115					120					125				
					CTT												432
	гåг	130	arg	ASP	Leu	File	ьуs 135	ьeu	TTG	ьeu	րչ	LуS 140	GIU	ASP	GIU	Leu	
50	GGG		AGA	тст	ATA	ATG		ACT	GTT	CAA	AAC		GAC				471
					Ile												411
	145				<del>-</del>	150					155						

(22) INFORMATION FOR SEQ ID NO:21: (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 471 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
5
            (ii) MOLECULE TYPE:cDNA
            (ix) FEATURE:
                (A) NAME/KEY: mat peptide
                (B) LOCATION: 1..471
                (C) IDENTIFICATION METHODS:S
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
10
     TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
                                                                            48
     Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
                                            10
     GAC CAA GTT CTC TTC ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT
                                                                            96
     Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
15
                   20
                                        25
     ATG ACT GAT TCT GAC TCT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT
     Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
              35
                                    40
                                                        45
     ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC
                                                                           192
20
     Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                               55
                                                    60
     TCT GTG AAG TCT GAG AAA ATT TCA ACT CTC TCC GCT GAG AAC AAA ATT
                                                                           240
     Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
                           70
                                                75
     ATT TCC TTT AAG GAA ATG AAT CCT CCT GAT AAC ATC AAG GAT ACA AAA
                                                                           288
25
     Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                                            90
     AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAT AAG
                                                                           336
     Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                  100
                                       105
                                                           110
     ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TCT GAA
                                                                           384
30
     Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                   120
              115
                                                       125
     AAA GAG AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG
                                                                           432
     Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                              135
                                                   140
35
     GGG GAT AGA TCT ATA ATG TTC ACT GTT CAA AAC GAA GAC
                                                                           471
     Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
                          150
      (23) INFORMATION FOR SEQ ID NO:22:
            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 471 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE:cDNA
            (iX) FEATURE:
                (A) NAME/KEY: mat peptide
                (B) LOCATION: 1..471
                (C) IDENTIFICATION METHODS: S
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
50
     AAC TTT GGC CGA CTT CAC GCT ACA ACC GCA GTA ATA CGG AAT ATA AAT
                                                                            48
     Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn
                                           10
     GAC CAA GTT CTC TTC GTT GAC AAA AGA CAG CCT GTG TTC GAG GAT ATG
                                                                            96
     Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
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				20					25				•	30			
	ACT Thr	GAT Asp	ATT Ile 35	GAT Asp	CAA Gln	AGT Ser	GCC Ala	AGT Ser 40	GAA Glu	CCC Pro	CAG Gln	ACC Thr	AGA Arg 45	CTG Leu	ATA Ile	ATA Ile	144
5	TAC Tyr	Met	TAC	AAA Lys	GAC Asp	AGT Ser	Glu	GTA	AGA Arg	GGA Gly	CTG Leu	Ala	GTG	ACC Thr	CTC Leu	TCT Ser	192
	Val	50 AAG Lys	GAT Asp	AGT Ser	AAA Lys	Met	55 TCT Ser	ACC Thr	CTC Leu	TCC Ser	Cys	60 AAG Lys	AAC Asn	AAG Lys	ATC Ile	ATT Ile	240
10	65 TCC Ser	TTT Phe	GAG Glu	GAA Glu	ATG Met	70 GAT Asp	CCA Pro	CCT Pro	GAA Glu	Asn	75 ATT Ile	GAT Asp	GAT Asp	ATA Ile	CAA Gln	80 AGT Ser	288
15	GAT Asp	CTC Leu	ATA Ile	Phe	85 TTT Phe	CAG Gln	AAA Lys	CGT Arg	Val	90 CCA Pro	GGA Gly	CAC His	AAC Asn	Lys	95 ATG Met	GAG Glu	336
	TTT Phe	GAA Glu	Ser	100 TCA Ser	CTG Leu	TAT Tyr	GAA Glu	Gly	105 CAC His	TTT Phe	CTT Leu	GCT Ala	Cys	110 CAA Gln	AAG Lys	GAA Glu	384
20	GAT Asp	Asp	115 GCT Ala	TTC Phe	AAA Lys	CTC Leu	Ile	120 CTG Leu	AAA Lys	AAA Lys	AAG Lys	Asp	125 GAA Glu	AAT Asn	GGG Gly	GAT Asp	432
	Lys				TTC Phe	Thr					His						471
25	145					150					155						
20	(24)				V FOR												
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30					PE : ni RANDI				<u> </u>								
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	AAC	TTT	GGC	CGA	CTT Leu	CAC	TGT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
40	1				5					10			_		15		2.4
	Asp	Gln	Val	Leu 20	TTC Phe	Val	Asp	Lys	Arg 25	Gln	Pro	Val	Phe	GAG Glu 30	Asp	Met	96
	ACT Thr	GAT	ATT	GAT	CAA Gln	AGT	GCC Ala	AGT	GAA	CCC	CAG	ACC	AGA	CTG	ATA	ATA	144
45			35		GAC			40					45				100
					Asp												192
	GTG Val	AAG	GAT	AGT	AAA Lys	ATG	TCT	ACC	CTC	TCC	TGT	AAG	AAC	AAG	ATC	ATT	240
50	65				ATG	70					75			•		80	200
	Ser	Phe	Glu	Glu	Met 85	Asp	Pro	Pro	Glu	Asn 90	Ile	Asp	Asp	Ile	Gln	Ser	288
5.5	GAT	CTC	ATA	TTC	TTT	CAG	AAA	CGT	GTT	CCA	GGA	CAC	AAC	AAG	95 ATG	GAG	336
55	voħ	neu	116	100	Phe	GIII	nys	Ary	105	110	GIÀ	пта	ASII	110	мес	GIU	

	111	GAA	101	LCH	CIG	IMI	GAA	GGA	CAC	1 1 1	C11	GCT	AGC	CAA	AAG	GAA	384
	Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Ser	Gln	Lys	Glu	301
	GAT	GAT	GCT	<b>TTC</b>	ΔΔΔ	CTC	ΔΤΤ		ααα	ΔΔΔ	AAC	CAT	C 2 2	ידית ה	GGG	CAT	432
5	Asp	Asp	Ala	Phe	Lys	Leu	Ile 135	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp	432
	AAA		GTA	ATG	TTC	ACT		ACT	AAC	TTA	САТ		λст				471
	Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser				4/1
	145					150					155						
10																	
15	(25)		i) SE( [] []	ATION QUENC A) LEN B) TYN C) STI	CE CI NGTH PE:n:	HARA :69   ucle	CTER: base ic a	ISTIC pain cid	CS: rs		٠						
			I) Li)MC LX)FI ()	D) TOI DLECT EATUI A) NAI	POLO JLE : RE: ME/KI	GY:1: CYPE EY:s:	inea: :Othe ig pe	r er nu	ucle	ic a	cid,						
20				3) LO( 3) IDI				METI	שחחב	. c			•				
		()		EQUE							0:24	:					
	ATG												GCTG	CAA (	GTCA	AGCTGC	60
25		GTGG															69
25																	
	(26)			OITA													
		(:		QUEN(													
30		•		3) TYI					LLS								
30			) I) Li) <b>M</b> (	C) STE D) TOE DLECU	RANDE POLOC JLE	EDNES SY:1: CYPE	SS:do inea: :cDN/	ouble c	€								
		7)		RIGIN													
35				A) OR( 7) TIS				ær									
		( i	ix) fe	EATUI	RE:				ie								
			( E	3) LO	CATIO	ON:1	471	Ļ									
		1.		C) IDE							10 a r	_					
40		()	(1)31	rQOEI	NCE I	ESCI	CIPII	LON:	SEQ	ID I	VO:25	o:					
															TTG Leu 15		48
		CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA		CGG	CCT	СТА	TTT	GAA	GAT	96
45	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glu	Asp	
														ATA	TTT		144
	Met	Thr	Asp 35	Ser	Asp	Cys	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr 45	Ile	Phe	Ile	
50															ACT		192
		50					55			_		60			Thr		
															AAA		240
	Ser 65	val	ьуs	Cys	Glu	Lys 70	пе	Ser	Thr	Leu	Ser 75	Cys	Glu	Asn	Lys	Ile 80	
55		TCC	TTT	AAG	GAA		AAT	CCT	CCT	GAT		ATC	AAG	GAT	ACA		288
								Pro									

```
85
                                           90
     AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAT AAG
                                                                           336
     Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                  100
                                       105
                                                           110
     ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TGT GAA
                                                                           384
     Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
              115
                                  120
                                                       125
     AAA GAG AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG
     Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
         130
                              135
                                                   140
10
     GGG GAT AGA TCT ATA ATG TTC ACT GTT CAA AAC GAA GAC
                                                                           471
     Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
                          150
     (27) INFORMATION FOR SEQ ID NO:26:
           (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 570 base pairs
                (B) TYPE: nucleic acid
                (C) STRANDEDNESS: double
                (D) TOPOLOGY: linear
20
            (ii) MOLECULE TYPE: CDNA
            (iX) FEATURE:
                (A) NAME/KEY:5' UTR
                (B) LOCATION: 1..15
                (C) IDENTIFICATION METHODS:S
25
                (A) NAME/KEY: sig peptide
                (B) LOCATION: 16..84
                (C) IDENTIFICATION METHODS:S
                (A) NAME/KEY: mat peptide
                (B) LOCATION: 85..555
                (C) IDENTIFICATION METHODS: S
30
                (A) NAME/KEY: 3' UTR
                (B) LOCATION: 556..570
                (C) IDENTIFICATION METHODS:S
            (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
35
     ACACCTCGAG CCACC ATG GCC TTG ACC TTT GCT TTA CTG GTG GCC CTC CTG
                       Met Ala Leu Thr Phe Ala Leu Leu Val Ala Leu Leu
                                   -20
     GTG CTC AGC TGC AAG TCA AGC TGC TCT GTG GGC TAC TTT GGC AAG CTT
     Val Leu Ser Cys Lys Ser Ser Cys Ser Val Gly Tyr Phe Gly Lys Leu
                              - 5
40
     GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT GAC CAA GTT CTC TTC
                                                                           147
     Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe
                                          15
     ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT ATG ACT GAT TCT GAC
     Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp
                                      30
45
     TGT AGA GAT AAT GCA CCC CGG ACC ATA TTT ATT ATA AGT ATG TAT AAA
                                                                           243
     Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys
              40
                                  45
     GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC TCT GTG AAG TGT GAG
                                                                           291
     Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Cys Glu
50
                              60
                                                   65
     AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT ATT TCC TTT AAG GAA
                                                                           339
     Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu
                          75
                                               80
     ATG AAT CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC ATC ATA TTC
     Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe
                      90
```

	TTT Phe	CAG Gln	AGA Arg	AGT Ser 105	GTC Val	CCA Pro	GGA Gly	CAT His	GAT Asp 110	AAT Asn	AAG Lys	ATG Met	CAA Gln	TTT Phe	GAA Glu	TCT Ser	435
5	TCA Ser	TCA Ser	TAC Tyr 120	GAA	GGA Gly	TAC Tyr	TTT Phe	CTA Leu 125	GCT Ala	TGT Cys	GAA Glu	AAA Lys	GAG Glu 130	AGA	GAC Asp	CTT Leu	483
	TTT Phe	AAA Lys 135	CTC	ATT Ile	TTG Leu	AAA Lys	AAA Lys 140	GAG	GAT Asp	GAA Glu	TTG Leu	GGG Gly 145	GAT	AGA Arg	TCT Ser	ATA Ile	531
10		TTC			CAA Gln		GAA		TAGO	GCGG	CCG (		r				570
15	(28)	(:	i) SE( () () ()	QUENC A) LEI B) TYI O) TOI	N FOR CE CH NGTH PE:ar POLOC	HARAC :10 a nino GY:li	TERI amino acio inear	ISTIC o aci i	CS:								
20		7)	v) FRI	AGME	JLE T NT TY NCE I	PE:	V-te	cmina		_		:					
	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10							
25	/ 0.01								_								
	(29)		i)SE( // //	QUENC A) LEI B) TYI	N FOI CE CH NGTH: PE:nu	HARAC :471 :clei	Dase base	STIC pai	CS: irs		٠						
30			1) ii)M( vi)O! ()	O) TOI OLECT RIGII A) ORO	RANDI POLOC JLE 1 NAL 3 SANIS SSUE	SY:li TYPE: SOUR( SM:mc	inean :cDN/ CE: ouse	A A	9								
35			ix) fi () () ()	EATUI A) NAM B) LOC C) IDI		EY:ma DN:1. FICAT	at pe	eptic L METH	HODS :		√O : 28	3:					
40					CTT Leu 5												48
45					TTC Phe												96
				GAT	CAA Gln				GAA					CTG			144
50			TAC		GAC Asp			GTA					GTG				192
		AAG			AAA Lys							AAG					240
55	TCC				ATG Met 85											AGT	288

							-			•							
	GAT Asp	CTC Leu	ATA Ile	TTC Phe 100	TTT Phe	CAG Gln	AAA Lys	CGT Arg	GTT Val 105	CCA Pro	GGA Gly	CAC His	AAC Asn	AAG Lys 110	ATG Met	GAG Glu	336
5	TTT Phe	GAA Glu	TCT Ser 115	TCA	CTG Leu	TAT Tyr	GAA Glu	GGA Gly 120	CAC	TTT Phe	CTT Leu	GCT Ala	TGC Cys 125	CAA	AAG Lys	GAA Glu	384
	GAT Asp	GAT Asp 130	GCT	TTC Phe	AAA Lys	CTC Leu	ATT Ile 135	CTG Leu	AAA Lys	AAA Lys	AAG Lys	GAT Asp 140	GAA	AAT Asn	GGG Gly	GAT Asp	432
10		TCT					CTC	ACT Thr				CAA					471
15	(30)		L) SE( [] (E) ((	QUENC A) LEI B) TYI C) STI	CE CH NGTH: PE:nu RANDI	LARAC 570 clei	DTERI base ic ac	ouble	CS: irs								
20			Li)MC LX)FE (1	OLECT EATUR A) NAM B) LOC	ME/KI	TYPE: EY:5' ON:1.	CDN UTI	A.	פחטני	. c							
25			(2 (E (2 (E	A) NAM B) LOC C) IDM A) NAM B) LOC	ME/KI CATIO ENTII ME/KI CATIO	EY:si ON:16 FICAT EY:ma ON:85	ig pe 584 FION at pe 555	eption  METH  eption  55	de HODS: de	: S							
30		(2	( <i>I</i> (I	A) NAM B) LOC C) IDE	ME/KI CATIO ENTIE	EY:3' ON:59 FICAT	UTI 565 TION		HODS :	: S	10 : 2 <u>9</u>	€:					
35	ACA	CCTCC	GAG (	CCAC					r Phe					Ala		C CTG 1 Leu	51
	Val	Leu -10	Ser	Cys	Lys	Ser	Ser -5	TGC Cys	Ser	Val	Gly	Asn 1	Phe	Gly	Arg	Leu 5	. 99
40	His	Cys	Thr	Thr	Ala 10	Val	Ile	CGG Arg	Asn	Ile 15	Asn	Asp	Gln	Val	Leu 20	Phe	147
	Val	Asp	Lys	Arg 25	Gln	Pro	Val	TTC	Glu 30	Asp	Met	Thr	Asp	Ile 35	Asp	Gln	195
45	Ser	Ala	Ser 40	Glu	Pro	Gln	Thr	AGA Arg 45	Leu	Ile	Ile	Tyr	Met 50	Tyr	Lys	Asp	243
50	Ser	Glu 55	Val	Arg	Gly	Leu	Ala 60	GTG Val	Thr	Leu	Ser	Val 65	Lys	Asp	Ser	Lys	291
<i>50</i>	Met 70	Ser	Thr	Leu	Ser	Cys 75	Lys	AAC Asn	Lys	Ile	Ile 80	Ser	Phe	Glu	Glu	Met 85	339
<i>55</i>								GAT Asp									387
	CAG	AAA	CGT	GTT	CCA	GGA	CAC	AAC	AAG	ATG	GAG	TTT	GAA	TCT	TCA	CTG	435

```
Gln Lys Arg Val Pro Gly His Asn Lys Met Glu Phe Glu Ser Ser Leu
                  105
                                       110
     TAT GAA GGA CAC TTT CTT GCT TGC CAA AAG GAA GAT GAT GCT TTC AAA
                                                                            483
     Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu Asp Asp Ala Phe Lys
5
              120
                                   125
                                                        130
     CTC ATT CTG AAA AAA AAG GAT GAA AAT GGG GAT AAA TCT GTA ATG TTC
                                                                            531
     Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp Lys Ser Val Met Phe
                               140
                                                    145
     ACT CTC ACT AAC TTA CAT CAA AGT TAGGCGGCCG CGTGT
                                                                            570
10
     Thr Leu Thr Asn Leu His Gln Ser
     150
                           155
      (31) INFORMATION FOR SEO ID NO:30:
            (i) SEQUENCE CHARACTERISTICS:
15
                (A) LENGTH:6 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear
            (ii) MOLECULE TYPE:peptide
            (v) FRAGMENT TYPE: N-terminal fragment
```

Asn Phe Gly Arg Leu His

Claims

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An isolated polypeptide which is capable of inducing the production of interferon-gamma by immunocompetent
cells, said polypeptide containing either amino acid sequence wherein one or more cysteines are replaced with
different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs:1-3 intact, or that
wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the
consensus sequences but excluding those of the replaced cysteine(s);

SEQ ID NO: 1:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Asn Asp Gln Val Leu Phe 1 5

SEO ID NO: 2:

Phe Glu Asp Met Thr Asp
1 5

SEQ ID NO: 3:

Met Tyr Lys Asp Ser.
1 5

- 2. The polypeptide of claim 1, wherein said different amino acid(s) is one or more amino acids selected from the group consisting of serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine.
- 55 3. The polypeptide of claim 1, wherein the amino acid sequence wherein one or more cysteines are replaced with deferent amino acid(s) is derived from the amino acid sequence of SEQ ID NO:4, containing SEQ ID NOs:1-3 as consensus sequences;

### SEQ ID NO: 4:

5	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn
	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glu	Asp
10	Met	Thr	Asp 35	Ser	Asp	Cys	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr 45	Ile	Phe	Ile
	Ile	Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile
	Ser 65	Val	Lys	Cys	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Cys	Glu	Asn	Lys	Ile 80
15	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys
	Ser	Asp	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys
20	Met	Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu
	Lys	Glu 130	Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu	Asp	Glu	Leu
25	Gly 145	Asp	Arg	Ser	Ile	Met 150	Phe	Thr	Val	Gln	Asn 155	Glu	Asp.	•		•

4. The polypeptide of claim 1, wherein the amino acid sequence wherein one or more cysteines replaced with different amino acid(s) is derived from the amino acid sequence of SEQ ID NO:5, containing SEQ ID NO:1-3 as consensus sequences;

### SEQ ID NO: 5:

30

35	Asn 1	Phe	Gly	Arg	Leu 5	His	Cys	Thr	Thr	Ala 10	Val	Ile	Arg	Asn	Ile 15	Asn
33	Asp	Gln	Val	Leu 20	Phe	Val	Asp	Lys	Arg 25	Gln	Pro	Val	Phe	Glu 30	Asp	Met
	Thr	Asp	Ile 35	Asp	Gln	Ser	Ala	Ser 40	Glu	Pro	Gl'n	Thr	Arg 45	Leu	Ile	Ile
40	Tyr	Met 50	Tyr	Lys	Asp	Ser	Glu 55	Val	Arg	Gly	Leu	Ala 60	Val	Thr	Leu	Ser
	Val 65	Lys	Asp	Ser	Lys	Met 70	Ser	Thr	Leu	Ser	Cys 75	Lys	Asn	Lys	Ile	Ile 80
45	Ser	Phe	Glu	Glu	Met 85	Asp	Pro	Pro	Glu	Asn 90	Ile	Asp	Asp	Ile	Gln 95	Ser
	Asp	Leu	Ile	Phe 100	Phe	Gln	Lys	Arg	Val 105	Pro	Gly	His	Asn	Lys 110	Met	Glu
	Phe	Glu	Ser 115	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Cys 125	Gln	Lys	Glu
50	Asp	Asp 130	Ala	Phe	Lys	Leu	Ile 135	Leu	Lys	Lys	Lys	Asp 140	Glu	Asn	Gly	Asp
	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser.			

5. The polypeptide of claim 1, which contains an amino acid sequence selected from the group consisting of SEQ ID NO:4;

SEQ ID NO: 6:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 10 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 15 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 20 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

#### 25 SEQ ID NO: 7:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 30 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 35 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 40 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 45 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 145 150

#### SEQ ID NO: 8:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile

```
35
                                      40
        Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
5
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                    100
                                         105
10
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                     120
        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                                 135
        Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
15
                            150
        SEQ ID NO: 9:
        Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
20
                                             10
        Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                                         25
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
25
                                 55
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
                                              90
30
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                                         105
                    100
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                    120
        Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
35
                                135
        Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
                            150
        SEQ ID NO: 10:
40
        Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
        Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
                                         2.5
45
        Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
        Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
        Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile
50
                                                 75
        Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
        Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                                        105
        Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
5<u>5</u>
                115
```

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Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                              135
     Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
                          150
     SEQ ID NO: 11:
     Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
10
     Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
     Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
     Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                              55
     Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
                                              .75
     Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
20
     Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                                      105
     Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
                                  120
25
     Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                              135
     Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
     145
                          150
30
     SEQ ID NO: 12:
     Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
                                          10
     Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
35
     Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
     Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
                              55
40
     Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile
                          70
                                              75
     Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
     Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
                                      105
     Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu
                                  120
     Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
                              135
50
     Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp.
     145
                         150
                                              155
```

The polypeptide of claim 1, which contains an amino acid sequence selected from the group consisting of SEQ
 ID NOs:13 and 14, derived from the amino acid sequence of SEQ ID NO:5;

SEQ ID NO: 13:

- Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn 5 10 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 25 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile 40 10 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 75 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 15 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu 115 120 20 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 135 140 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 150 155 145
- 25 SEQ ID NO: 14:
- Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 30 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 55 35 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 75 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 90 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 40 100 105 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Ser Gln Lys Glu 120 115 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 140 135 45 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser. 150 155 145
- 7. The polypeptide of claims 1, which additionally has one or more properties selected from the group consisting of enhancing cytotoxicities of killer cells and inducing formation of killer cells.
  - 8. The polypeptide of claim 3, which additionally has one or more properties selected from the group consisting of enhancing cytotoxicity of killer cells and inducing formation of killer cells.
- 9. A DNA encoding the polypeptide of claim 1.
  - 10. The DNA of claim 9, which contains a nucleotide sequence selected either from the group consisting of the nucleotide sequences of SEQ ID NOs:15-23 and their complementary nucleotide sequences, or from the other group

consisting of the nucleotide sequences derived from one of the nucleotide sequences of the former group by replacing one or more of the nucleotides with different one(s) without altering the amino acid sequences encoded thereby;

	SEQ	ID !	10:	15:									
10					CTT Leu 5								 48
,,					TTC Phe								96
15					GAC Asp								144
				_	AAA Lys								 192
20	TCT Ser 65		_	-	GAG Glu		-			-	_	-	 240
					GAA Glu 85								288
25					TTC Phe			_					336
					TCT								384
30					CTT Leu								432
35		GAT			ATA Ile								471

SEQ ID NO: 16:

5	Tyr 1	Phe	Gly	.Lys	CTT Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn	48
	Asp	Gln	Val	leu 20	TTC Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glu	Asp	96
10					GAC Asp												144
					AAA Lys												192
15					GAG Glu												240
					GAA Glu 85												288
20					TTC Phe												336
25					TCT Ser												384
					CTT Leu												432
30					ATA												471
	SEQ	ID N	10: 1	17:													
35					CTT Leu 5												48
					TTC Phe												96
40					GAC Asp												144
					AAA Lys												192
45					GAG Glu												240
	ATT				GAA Glu 85						AAC					AAA	288
50					TTC Phe					GTC					AAT		336
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		Met	Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Ser	Glu	
		AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
5	;															Glu		
		GGG		AGA	TCT	ATA	ATG		ACT	GTT	CAA	AAC		GAC				471
				Arg														412
		145		- 3			150					155						
10	0	SEQ	ID 1	10:	18:													
		TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
		Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
		1				5					10			_		15		
13	5															GAA		96
		Asp	Gln	Val		Phe	Ile	Asp	Gln		Asn	Arg	Pro	Leu		Glu	Asp	
					20.					25					30			
																TTT		144
		Met	Thr		Ser	Asp	Ser	Arg		Asn	Ala	Pro	Arg		Ile	Phe	Ile	
_	_			35	<b></b>		~~~		40			~~~		45				
20	o															ACT		192
		TTE		met	TYT	răs	ASp		Gin	PIO	arg	GTA		ATa	vaı	Thr	11e	
		m.c.m	50	330	mem	CAC	* * *	55	mc »	N COM	CmC	mcc	60 mcm	010			3 mm	240
																AAA		240
			AGT	гĀг	Ser	GIU		TIE	Ser	TIME	ьeu	75	Cys	GIU	ASII	Lys		
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	•						_									Thr		200
		110			<b>-</b> 17	85	1.00		110		90		140	2,3	wab	95	Lys	
	•	AGT	GAC	ATC	ATA		ттт	CAG	AGA	AGT		CCA	GGA	CAT	GAT	AAT	AAG	336
																Asn		-
30	o				100				<b> </b>	105			2		110		-4-	
		ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TCT	GAA	384
																Ser		
				115					120			_		125				
																GAA		432
3.	_	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
٥.	3		130					135					140		٠.			
				AGA														471
			Asp	Arg	Ser			Phe	Thr	Val	Gln		Glu	Asp				
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		1	1110	Gry	nys	5	GIU	Ser	Буз	пеи	10	AGI	110	ALY	nan	15	USII	
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4	5															Glu		30
		p	04	142	20	1110	110	ngp	01	25		9	• • •	acu.	30	Olu	NSD	
		ATG	ACT	GAT		GAC	ጥርጥ	AGA	GAT		GCA	CCC	CGG	ACC		TTT	ጥጥል	144
																Phe		
				35				9	40			,	3	45				
5	0	АТА	AGT		TAT	AAA	GAT	AGC		CCT	AGA	GGT	ATG		GTA	ACT	ATC ·	192
-	-															Thr		
			50		_	_	-	55			_		60					
		TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TCT	GAG	AAC	AAA	ATT	240

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		Val	Lys	Ser	Glu		Ile	Ser	Thr	Leu		Ser	Glu	Asn	Lys		
	65 איזייני א	TCC	ጥጥጥ	AAC	CAA	70 atro	ልልጥ	CCT	CCm	C እ T	75	እ ጥ C	A A C	CAM	A C A	80	288
5		Ser															200
3				-1 -	85					90			-, -		95	-,0	
		GAC															336
	Ser	Asp	Ile		Phe	Phe	Gln	Arg		Val	Pro	Gly	His	-	Asn	Lys	
	1 mc	~	mmm	100	mcm.	mc x	ma x	m > 0	105	003	m > 0	mmm		110			
10		CAA Gln															384
	Mec	GIU	115	GIU	Ser	261	ser	120	GIU	GIY	TAT	FILE	125	WIG	ser	GIU	
	AAA	GAG		GAC	CTT	TTT	AAA		ATT	TTG	AAA	AAA		GAT	GAA	TTG	432
		Glu															
		130					135					140		_			
15		GAT															471
	-	Asp	Arg	Ser	He		Phe	Thr	Val	Gin		Glu	Asp				
	145		•			150					155						
	SEQ	ID I	10: 3	20:													
	-																
20		TTT															48
		Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu		Val	Ile	Arg	Asn		Asn	
	CVC T	CAA	Contro	רייר	ውምር	አጥጥ	CAC	$C$ $\lambda$ $\lambda$	CCA	10 מיימג	ccc	ССТ	CTLY	ատա	15	Cam	96
		Gln															90
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20	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC.	ATA	TTT	ATT	144
	Met	Thr		Ser	Asp	Ser	Arg		Asn	Ala	Pro	Arg		Ile	Phe	Ile	
	3 m 3	3 Cm	35	m a m		0 N M	100	40	O C M		o c m	) MC	45	Omn		<b>3</b> mo	100
		AGT Ser															192
30	110	50	1100	. , .	Lys	ngp	55	0111	110	y	Ory	60	YTO	V G T	1111	116	
	TCT	GTG	AAG	TCT	GAG	AAA		TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
		Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	_	Ala	Glu	Asn	Lys		
	65	mcc	mmm		C	70		com		C A m	75	3 m C		^~~		80	200
		TCC Ser															288
35	110	001		ט גַט	85				110	90	*****		ט עַנו	nsp	95	ny 3	
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT		AAG	336
	Ser	Asp	Ile		Phe	Phe	Gln	Arg		Val	Pro	Gly	His	Asp	Asn	Lys	
				100		~~-			105					110			
40		CAA Gln															384
•••	Met	9111	115	Giu	Ser	Ser	Ser	120	GIU	GLY	ıyı	FILE	125	YIG	Cys	GIU	
	AAA	GAG		GAC	CTT	TTT	AAA		ATT	TTG	AAA	AAA		GAT	GAA	TTG	432
		Glu															
•		130		•			135					140					
45		GAT															471
	145	Asp	Arg	Ser	TTE	met 150	Pne	Thr	vaı	GIN	155	GIU	Asp				
	140					130					133						
	SEQ	ID N	10: 2	21:													
	_																
50		TTT															48
		Phe	Gly	Lys		Glu	Ser	Lys	Leu	_	Val	He	Arg	Asn		Asn	
	CAC	CAA	Crim	כיייכ	ттС -	አጥጥ	GAC	ממר	CCA	10 ልልጥ	CGG	CCT	СТА	արդիսի	15 GAA	CAM	0.6
	GAC	CAA	GII	CIC	110	VII	UNC	CAA	GGA	WWI	CGG		CIA	111	GAA	GAT	96

	Asp	Gln	Val	Leu 20		Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30		Asp	
	ATG	ACT	GAT		GAC	TCT	AGA	GAT		GCA	CCC	CGG	ACC			АТТ	144
5	Met	Thr	Asp 35	Ser	Asp	Ser	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr 45	Ile	Phe	Ile	433
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
		50		-	Lys	-	55			_	_	60				_	
10					GAG												240
10		Val	Lys	Ser	Glu		Ile	Ser	Thr	Leu		Ala	Glu	Asn	Lys	Ile	
	65	maa				70				~~~	75					80	
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
					Glu 85					90				_	95	-	
15					TTC												336
				100	Phe				105					110		-	
					TCT												384
	Met	GIII	115	GIU	Ser	ser	ser	120	ĢΙU	GIÀ	Tyr	Pne	125	ATS	Ser	Glu	
20	AAA	GAG		GAC	CTT	TTT	AAA		ATT	TTG	AAA	AAA		GAT	GAA	TTG	432
					Leu												
	GGG		AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
					Ile												-, -
25	145					150					155				•		
•	SEQ	ID i	NO: 2	22:													
	AAC	TTT	GGC	CGA	CTT	CAC	GCT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
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30	1			_	5					10			_		Ile 15		
30	1 GAC	CAA	GTT	CTC	5 TTC	GTT	GAC	AAA	AGA	10 CAG	CCT	GTG	TTC	GAG	15 GAT	ATG	96
30	1 GAC Asp	CAA Gln	GTT Val	CTC Leu 20	5 TTC Phe	GTT Val	GAC Asp	AAA Lys	AGA Arg 25	10 CAG Gln	CCT Pro	GTG Val	TTC Phe	GAG Glu 30	15 GAT Asp	ATG Met	
	1 GAC Asp ACT	CAA Gln GAT	GTT Val	CTC Leu 20 GAT	5 TTC Phe CAA	GTT Val AGT	GAC Asp GCC	AAA Lys AGT	AGA Arg 25 GAA	10 CAG Gln CCC	CCT Pro	GTG Val ACC	TTC Phe	GAG Glu 30 CTG	15 GAT Asp ATA	ATG Met	96 144
35	1 GAC Asp ACT Thr	CAA Gln GAT Asp	GTT Val ATT Ile 35	CTC Leu 20 GAT Asp	5 TTC Phe CAA Gln	GTT Val AGT Ser	GAC Asp GCC Ala	AAA Lys AGT Ser 40	AGA Arg 25 GAA Glu	10 CAG Gln CCC Pro	CCT Pro CAG Gln	GTG Val ACC Thr	TTC Phe AGA Arg 45	GAG Glu 30 CTG Leu	15 GAT Asp ATA Ile	ATG Met ATA Ile	144
	GAC Asp ACT Thr	CAA Gln GAT Asp	GTT Val ATT Ile 35 TAC	CTC Leu 20 GAT Asp	5 TTC Phe CAA Gln GAC	GTT Val AGT Ser	GAC Asp GCC Ala GAA	AAA Lys AGT Ser 40 GTA	AGA Arg 25 GAA Glu AGA	10 CAG Gln CCC Pro	CCT Pro CAG Gln CTG	GTG Val ACC Thr	TTC Phe AGA Arg 45 GTG	GAG Glu 30 CTG Leu	15 GAT ASP ATA Ile CTC	ATG Met ATA Ile	
	GAC Asp ACT Thr	CAA Gln GAT Asp ATG Met	GTT Val ATT Ile 35 TAC	CTC Leu 20 GAT Asp	5 TTC Phe CAA Gln	GTT Val AGT Ser	GAC Asp GCC Ala GAA Glu	AAA Lys AGT Ser 40 GTA	AGA Arg 25 GAA Glu AGA	10 CAG Gln CCC Pro	CCT Pro CAG Gln CTG	GTG Val ACC Thr GCT Ala	TTC Phe AGA Arg 45 GTG	GAG Glu 30 CTG Leu	15 GAT ASP ATA Ile CTC	ATG Met ATA Ile	144
35	GAC Asp ACT Thr TAC	CAA Gln GAT Asp ATG Met 50	GTT Val ATT Ile 35 TAC	CTC Leu 20 GAT Asp AAA Lys	5 TTC Phe CAA Gln GAC Asp	GTT Val AGT Ser AGT Ser	GAC Asp GCC Ala GAA Glu 55	AAA Lys AGT Ser 40 GTA Val	AGA Arg 25 GAA Glu AGA Arg	10 CAG Gln CCC Pro GGA Gly	CCT Pro CAG Gln CTG Leu	GTG Val ACC Thr GCT Ala 60	TTC Phe AGA Arg 45 GTG Val	GAG Glu 30 CTG Leu ACC	15 GAT Asp ATA Ile CTC Leu	ATG Met ATA Ile TCT Ser	144
	GAC Asp ACT Thr TAC Tyr GTG	CAA Gln GAT Asp ATG Met 50 AAG	GTT Val ATT Ile 35 TAC Tyr	CTC Leu 20 GAT Asp AAA Lys	5 TTC Phe CAA Gln GAC	GTT Val AGT Ser AGT Ser	GAC Asp GCC Ala GAA Glu 55 TCT	AAA Lys AGT Ser 40 GTA Val	AGA Arg 25 GAA Glu AGA Arg	10 CAG Gln CCC Pro GGA Gly TCC	CCT Pro CAG Gln CTG Leu	GTG Val ACC Thr GCT Ala 60 AAG	TTC Phe AGA Arg 45 GTG Val	GAG Glu 30 CTG Leu ACC Thr	15 GAT ASP ATA Ile CTC Leu	ATG Met ATA Ile TCT Ser ATT	144
35	GAC Asp ACT Thr TAC Tyr GTG Val 65	CAA Gln GAT Asp ATG Met 50 AAG Lys	GTT Val ATT Ile 35 TAC Tyr GAT Asp	CTC Leu 20 GAT Asp AAA Lys AGT Ser	5 TTC Phe CAA Gln GAC Asp	GTT Val AGT Ser AGT Ser ATG Met 70	GAC Asp GCC Ala GAA Glu 55 TCT Ser	AAA Lys AGT Ser 40 GTA Val ACC Thr	AGA Arg 25 GAA Glu AGA Arg CTC Leu	10 CAG Gln CCC Pro GGA Gly TCC Ser	CCT Pro CAG Gln CTG Leu TGT Cys 75	GTG Val ACC Thr GCT Ala 60 AAG Lys	TTC Phe AGA Arg 45 GTG Val AAC Asn	GAG Glu 30 CTG Leu ACC Thr	15 GAT Asp ATA Ile CTC Leu ATC Ile	ATG Met ATA Ile TCT Ser ATT Ile 80	144
35	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC	CAA Gln GAT Asp ATG Met 50 AAG Lys	GTT Val ATT Ile 35 TAC Tyr GAT Asp	CTC Leu 20 GAT Asp AAA Lys AGT Ser	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met	GTT Val AGT Ser AGT Ser ATG Met 70 GAT	GAC Asp GCC Ala GAA Glu 55 TCT Ser	AAA Lys AGT Ser 40 GTA Val ACC Thr	AGA Arg 25 GAA Glu AGA Arg CTC Leu	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT Asn	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT	GTG Val ACC Thr GCT Ala 60 AAG Lys	TTC Phe AGA Arg 45 GTG Val AAC ASD	GAG Glu 30 CTG Leu ACC Thr AAG Lys	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln	ATG Met ATA Ile TCT Ser ATT Ile 80 AGT	144 192 240
<i>35</i>	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser	CAA Gln GAT Asp ATG Met 50 AAG Lys	GTT Val ATT Ile 35 TAC Tyr GAT Asp GAG Glu	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85	GTT Val AGT Ser AGT Ser ATG Met 70 GAT Asp	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro	AAA Lys AGT Ser 40 GTA Val ACC Thr	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT Asn 90	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp	TTC Phe AGA Arg 45 GTG Val AAC ASD	GAG Glu 30 CTG Leu ACC Thr AAG Lys	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95	ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser	144 192 240 288
35	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe	GTT Val ATT Ile 35 TAC Tyr GAT Asp GAG Glu	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT	GTT Val AGT Ser AGT Ser ATG Met 70 GAT ASP	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT ASN 90 CCA	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp	GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG	ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser GAG	144 192 240
<i>35</i>	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe	GTT Val ATT Ile 35 TAC Tyr GAT Asp GAG Glu	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT Phe	GTT Val AGT Ser AGT Ser ATG Met 70 GAT ASP	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT ASN 90 CCA	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp	GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG	ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser GAG	144 192 240 288
<i>35</i>	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT Asp	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe CTC Leu GAA	GTT Val ATT Ile 35 TAC TYr GAT Asp GAG Glu ATA Ile	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu TTC Phe 100 TCA	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT Phe	GTT Val AGT Ser AGT Ser ATG Met 70 GAT Asp CAG Gln	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro AAA Lys GAA	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro CGT Arg	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu GTT Val 105 CAC	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT ASN 90 CCA Pro	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile GGA Gly	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp CAC His	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT ASP AAC ASC	GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile AAG Lys 110 CAA	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG Met	ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser GAG Glu	144 192 240 288
<i>35</i>	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT Asp	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe CTC Leu GAA	GTT Val ATT Ile 35 TAC TYr GAT Asp GAG Glu ATA Ile	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu TTC Phe 100 TCA	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT Phe	GTT Val AGT Ser AGT Ser ATG Met 70 GAT Asp CAG Gln	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro AAA Lys GAA	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro CGT Arg GGA Gly	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu GTT Val 105 CAC	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT ASN 90 CCA Pro	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile GGA Gly	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp CAC His	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp AAC Cys	GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile AAG Lys 110 CAA	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG Met	ATG Met ATA Ile TCT Ser ATT Ile 80 AGT Ser GAG Glu	144 192 240 288 336
<i>35</i>	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT Asp TTT Phe	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe CTC Leu GAA Glu	GTT Val ATT Ile 35 TAC TYr GAT Asp GAG Glu ATA Ile TCT Ser 115	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu TTC Phe 100 TCA Ser	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT Phe CTG Leu	GTT Val AGT Ser AGT Ser ATG Met 70 GAT Asp CAG Gln TAT	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro AAA Lys GAA Glu	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro CGT Arg GGA Gly 120	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu GTT Val 105 CAC His	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT ASN 90 CCA Pro	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile GGA Gly CTT Leu	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp CAC His GCT Ala	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT ASP AAC Cys 125	GAG Glu 30 CTG Leu ACC Thr AAG Lys ATA Ile AAG Lys 110 CAA Gln	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG Met AAG Lys	ATG Met  ATA Ile  TCT Ser  ATT Ile 80 AGT Ser  GAG Glu  GAA Glu	144 192 240 288 336 384
35 40 45	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT Asp TTT Phe GAT	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe CTC Leu GAA Glu	GTT Val ATT Ile 35 TAC TYr GAT Asp GAG Glu ATA Ile TCT Ser 115 GCT	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu TTC Phe 100 TCA Ser	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT Phe CTG Leu	GTT Val AGT Ser AGT Ser ATG Met 70 GAT Asp CAG Gln TAT Tyr	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro AAA Lys GAA Glu ATT	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro CGT Arg GGA Gly 120 CTG	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu GTT Val 105 CAC His	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT Asn 90 CCA Pro TTT Phe AAA	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile GGA Gly CTT Leu AAG	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp CAC His GCT Ala GAT	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp AAC Cys 125 GAA	GAG Glu 30 CTG Leu ACC Thr AAG Lys 110 CAA Gln	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG Met AAG Lys	ATG Met  ATA Ile  TCT Ser  ATT Ile 80 AGT Ser  GAG Glu  GAA Glu  GAT	144 192 240 288 336
35 40 45	GAC Asp ACT Thr TAC Tyr GTG Val 65 TCC Ser GAT Asp TTT Phe GAT	CAA Gln GAT Asp ATG Met 50 AAG Lys TTT Phe CTC Leu GAA Glu	GTT Val ATT Ile 35 TAC TYr GAT Asp GAG Glu ATA Ile TCT Ser 115 GCT	CTC Leu 20 GAT Asp AAA Lys AGT Ser GAA Glu TTC Phe 100 TCA Ser	5 TTC Phe CAA Gln GAC Asp AAA Lys ATG Met 85 TTT Phe CTG Leu	GTT Val AGT Ser AGT Ser ATG Met 70 GAT Asp CAG Gln TAT Tyr	GAC Asp GCC Ala GAA Glu 55 TCT Ser CCA Pro AAA Lys GAA Glu ATT	AAA Lys AGT Ser 40 GTA Val ACC Thr CCT Pro CGT Arg GGA Gly 120 CTG	AGA Arg 25 GAA Glu AGA Arg CTC Leu GAA Glu GTT Val 105 CAC His	10 CAG Gln CCC Pro GGA Gly TCC Ser AAT Asn 90 CCA Pro TTT Phe AAA	CCT Pro CAG Gln CTG Leu TGT Cys 75 ATT Ile GGA Gly CTT Leu AAG	GTG Val ACC Thr GCT Ala 60 AAG Lys GAT Asp CAC His GCT Ala GAT	TTC Phe AGA Arg 45 GTG Val AAC Asn GAT Asp AAC Cys 125 GAA	GAG Glu 30 CTG Leu ACC Thr AAG Lys 110 CAA Gln	15 GAT Asp ATA Ile CTC Leu ATC Ile CAA Gln 95 ATG Met AAG Lys	ATG Met  ATA Ile  TCT Ser  ATT Ile 80 AGT Ser  GAG Glu  GAA Glu  GAT	144 192 240 288 336 384

•	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155		Ser				
5	SEQ	ID I	NO: :	23:													
		TTT Phe															48
10		CAA Gln															96
		GAT Asp															144
15		ATG Met 50								•							192
		AAG Lys					TCT										240
20	TCC	TTT Phe				GAT					ATT					AGT	288
		CTC Leu			TTT					CCA					ATG		. 336
25		GAA Glu		TCA					CAC					CAA			384
30		GAT Asp 130	GCT					CTG					GAA				432
		TCT Ser															471
35	11 Th		of clair	n a wi	nich co	ntains	the nu	clectic	امع ما	ionca	of SEC	א חו	7.24 2	t the 5	-termir	nie	

35 11. The DNA of claim 9, which contains the nucleotide sequence of SEQ ID NO:24 at the 5'-terminus.

SEQ ID NO: 24:

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ATGGCCTTGA CCTTTGCTTT ACTGGTGGCC CTCCTGGTGC TCAGCTGCAA GTCAAGCTGC 60
TCTGTGGGC 69

- 12. The DNA of claim 9, which is inserted into an autonomously replicable vector.
- 13. The DNA of claim 9, which is introduced into an appropriate host.
  - 14. The DNA of claim 13, wherein said host is a cell selected from the group consisting of mammalian-epithelial, interstitial, and -hematopoietic cells.
- 50 15. A process for producing a polypeptide, which comprises the steps of:

culturing a cell containing a DNA encoding the polypeptide of claim 1, obtainable by introducing the DNA into an appropriate host, to produce a polypeptide, and collecting the produced polypeptide from the resulting culture.

16. The process of claim 15, wherein said host is a cell selected from the group consisting of mammalian-epithelial, interstitial, and -hematopoietic cells.

- 17. The process of claim 15, wherein said polypeptide is collected by one or more techniques selected from the group consisting of dialysis, salting out, filtration, concentration, fractional precipitation, ion-exchange chromatography, isoelectric focusing chromatography, hydrophobic chromatography, reversed phase chromatography, affinity chromatography, gel electrophoresis, and isoelectric focusing gel electrophoresis.
- 18. The process of claim 15, wherein said polypeptide is collected by an immunoaffinity chromatography using a monoclonal antibody.
- 19. An agent for susceptive diseases, which contains the polypeptide of claim 1 as an effective ingredient.
- 20. The agent of claim 19, which additionally contains interleukin 2.
- 21. The agent of claim 19, which contains a serum albumin, gelatin, a saccharide, or a buffer as a stabilizer.
- 22. The agent of claim 19, which is in the form of an antitumor agent.
  - 23. The agent of claim 22, which is in the form of an antitumor immunotherapeutic agent.
  - 24. The agent of claim 19, which is in the form of an antiviral agent.
  - 25. The agent of claim 19, which is in the form of an antimicrobial agent.
  - 26. The agent of claim 19, which is in the form of an anti-immunopathic agent.
- 25 27. The agent of claim 26, which additionally contains interleukin 12.
  - 28. The agent of claim 26, which is for treating atopic diseases.

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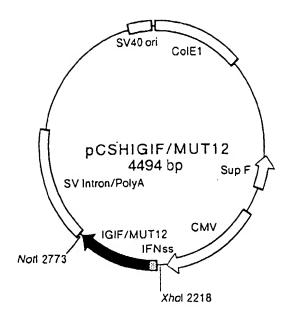
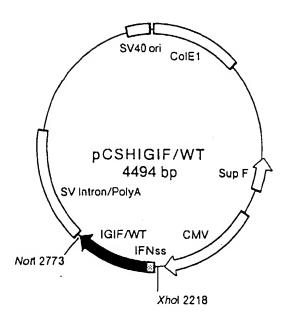
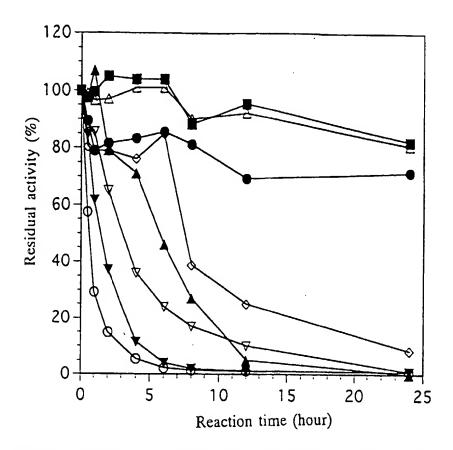


FIG. 1

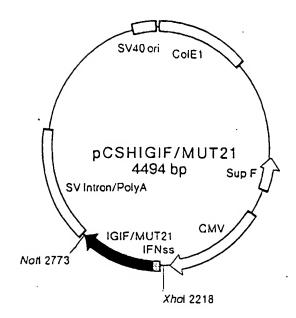


<u>FIG. 2</u>



Note: In the figure, the symbol "O—O" shows the time course upon the activity of a wild-type polypeptide; and the symbols "▼—▼", "▲—▲", "▼—▼", "♦—●", "■—■", and "△—△" show the time course upon the activity of the present polypeptides obtained by the methods in Examples A-1 to A-7, respectively.

<u>FIG. 3</u>



<u>FIG. 4</u>

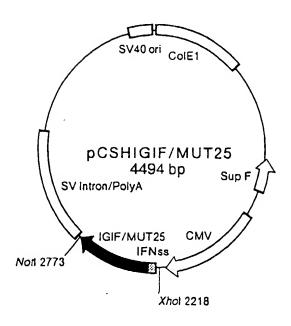
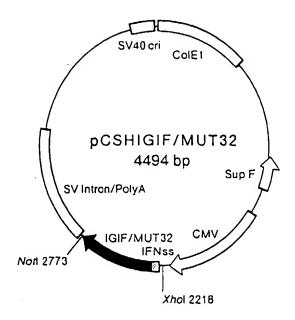


FIG. 5



<u>FIG. 6</u>

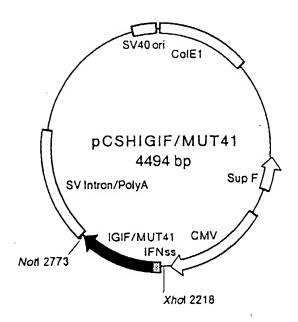


FIG. 7

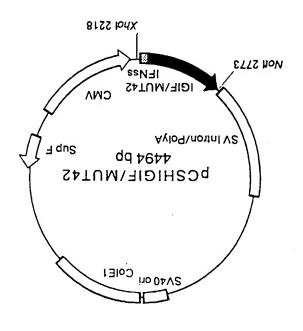
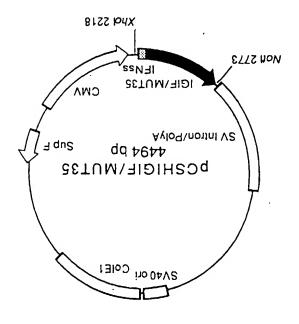


FIG. 8



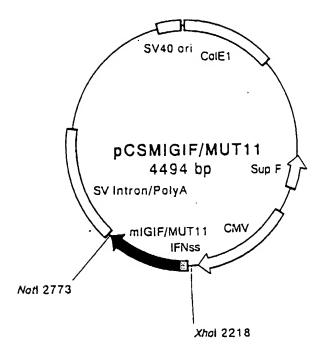


FIG. 10

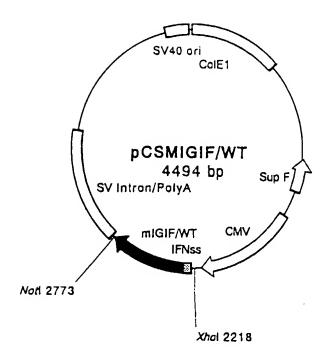
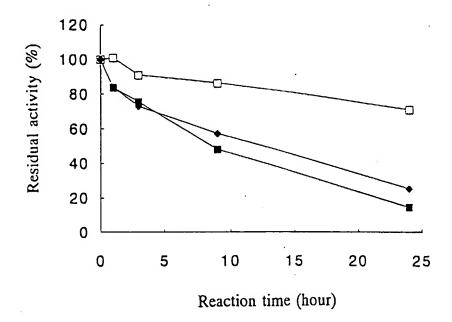


FIG. 11



Note: In the figure, the symbol " shows the time course upon the activity of a wild-type polypeptide; and the symbols " and " • • " show the time course upon the activity of the present polypeptides obtained by the methods in Examples A-8 and A-9, respectively.

FIG. 12

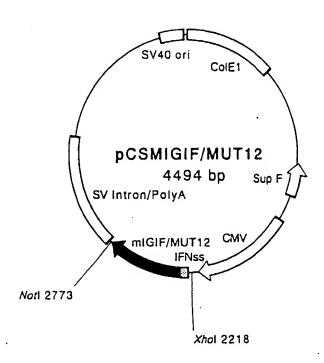


FIG. 13